INFLUENCE OF MYCORRHIZAS ON PLANT COMPETITION FOR PHOSPHORUS BETWEEN SLASH PINE AND GRASS

By

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To Karen,

for her love, support, patience

and her sense of humor that carried

us both through this adventure

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Individual plants benefit from the mycorrhizal condition primarily by improved nutrient uptake, especially of phosphorus (P), resulting in enhanced plant survival and growth in resource-limited conditions. On a broader scale, mycorrhizas have the potential to mediate plant competition and subsequently may be important at the community level.

In the southeastern United States, slash pine (*Pinus elliottii* Engelm. var. *elliottii*) is grown in plantations, where it competes for nutrients with grasses and other herbaceous vegetation. One objective of my research was to assess mycorrhizal contribution to intra- and interspecific plant competition for P in the greenhouse between pine and *Panicum chamaelonche* Trin., a dominant grass species at a local plantation site. The grass was inoculated or noninoculated with the arbuscular mycorrhizal (AM) fungus, *Glomus* sp. (INVAM FL329, formerly INVAM FL906), and pine was inoculated or

noninoculated with the ectomycorrhizal (EM) fungus, *Pisolithus tinctorius* (isolate S106). The effect of P (0.32, 3.23 or 32.26 μM) on competition also was analyzed in the greenhouse because resource abundance can affect the outcome of competition and inorganic P is limiting in pine plantations. Inoculated grasses were not colonized at the end of the experiment and were excluded from data analyses. When competing with grass, inoculated pine acquired more P and had a higher total dry weight than noninoculated pine. Grass shoot-P content was reduced at the 32.26-μM P level when grown with pine, irrespective of the pine inoculation treatment.

I evaluated the effects of benomyl over time in the field and at 0, 20, 60 and 150 kg benomyl ha^{-1} equivalent in the greenhouse. My objective was to determine if benomyl would be suitable for controlling AM but not EM fungi as part of a larger competition experiment involving pine and weeds. No effect was observed on pine in the greenhouse. Colonized root length of benomyl-treated *Zea mays* L. plants in the greenhouse remained static and the response was not dose-dependent. In contrast, colonization in the control plants increased over time. Minimal reduction of grass colonization was observed in the field, where limitations to effective control were ground cover, timing in relation to mycorrhizal development and benomyl application as a spray instead of as a soil drench.

CHAPTER 1
INTRODUCTION

Mycorrhizal contribution to plant nutrient uptake, especially phosphorus (P), has been extensively studied. Under nutrient-limiting conditions mycorrhizas are able to enhance plant nutrient uptake by various mechanisms, thereby ameliorating plant stress. Under these conditions plants compete for limited nutrients and mycorrhizas may modify the competitive interactions between plants. Little work has addressed the role of mycorrhizas in this area of plant synecology. The existing studies primarily deal with arbuscular mycorrhizal (AM) fungi and their contribution to plant interactions. Many of these studies investigated facilitative mycorrhizal plant associations, where mycorrhizal fungi transfer nutrients from one plant to another through common hyphal connections. Only one study addressed the function of ectomycorrhizal (EM) fungi in plant competition. No studies to date have evaluated the role of mycorrhizas in competitive interactions between AM and EM plants, which occurs frequently during succession. In the following chapter, I review the various aspects of mycorrhizal functioning in plant autoecology and synecology and I detail their role in plant competition.

Most previous studies have been performed under controlled conditions in the greenhouse without the influence of complex interactions of other environmental variables. When bringing mycorrhizal questions to the field, one of the more difficult problems is the creation of a suitable nonmycorrhizal control, since a majority of plants

are normally mycorrhizal. Fungicides can be useful in distinguishing mycorrhizal effects from other influences on plants in the field. The fungicide benomyl is selective in that it has inhibitory effects on AM but not on EM fungi. The selectivity of this fungicide would be useful in isolating the nutrient uptake mediated by AM fungi in AM and EM plant competition studies. As part of a larger competition study involving slash pine (*Pinus elliottii*) and weeds at a field site northwest of Gainesville, I tested benomyl in the field and in the greenhouse to determine if it would suitably control AM but not EM fungi (Chapter 3).

I addressed the direct effect of mycorrhizas on plant competition for P in greenhouse and growth chamber studies involving slash pine and *Panicum chamaelonche*, a dominant weed species at the field site. The main objectives of the greenhouse competition study, described in Chapter 4, were to determine if (i) mycorrhizas affect plant competition at the interspecific or intraspecific level, (ii) competition is dependent on soil nutrient concentration and (iii) competitive abilities are related to differences in P uptake kinetics. The goals of the growth chamber study, presented in the first section of the Appendix, were to assess (i) the contribution of mycorrhizal fungal hyphae to total plant P uptake and (ii) the competitive abilities of the AM and EM fungi with respect to each other.

CHAPTER 2
MYCORRHIZAS AND PLANT COMPETITION

Introduction

Over the past several decades the perception of mycorrhizas has evolved from viewing them as a unique biological phenomena to understanding them as integral parts of ecosystems. Much of the literature on mycorrhizas has addressed issues pertaining to single plants. More recently, there has been a growing tendency to evaluate the synecological consequences of the mycorrhizal association. The employment of techniques such as minirhizotrons (Lussenhop and Fogel, 1993), image analysis (Smith and Dickson, 1991), root-excluding screens and radioisotope labelling, among others, is redirecting the field to a broader scale of ecology dealing with plant interactions and community structure. The challenges faced during the next decade will be even more complex, with the increasing need to study multi-organismal assemblages and their functions at the ecosystem level. The next steps towards a more holistic view of mycorrhizal function will be determined by technological advances that will allow us to gain knowledge of how microbial systems fit together into a cohesive unit. This knowledge will provide us with a better understanding of the environment and how to best manage it in a sustainable manner.

Ecosystem studies necessitate an understanding of the functional associations of organisms with each other and with their environment. For plants, one of the main

biological interactions is competition. The term competition will be used here as the interaction between two organisms requiring the same limiting resource, which results in the decreased growth, survival or reproductive capacity of one of the two organisms. Plants mainly compete for light, water and nutrients. Physiological flexibility, within genetic constraints, allows plants to adapt to changes in resource availability. Physiological flexibility is enhanced by a plant's symbiotic relationship with mycorrhizal fungi. Modification in physiology can result in alterations of nutrient absorption capacity (Marschner and Dell, 1994) and water relations (Safir et al., 1972), as well as enhance light utilization and capture (Krishna et al., 1981). Increased tolerance or resistance to other environmental stresses, such as plant diseases (Rosendahl and Rosendahl, 1990; Schönbeck, 1978), high heavy metal concentrations (Denny and Wilkins, 1987) or xenobiotics (Donnelly et al., 1993), also have been found in mycorrhizal plants. Although the vast majority of studies with mycorrhizas has been conducted with terrestrial, mycorrhizas also have been found in wetland plants and may function in nutrient uptake in vascular aquatic plants (Rickerl et al., 1994; Wigand and Stevenson, 1994).

The objective of this chapter is to review mycorrhizal effects on plant competition and community structure. However, to prepare the foundation for the synecology of the system, a review of the autecology of mycorrhizal plants is also presented.

The Autecology of the Mycorrhizal Symbiosis

Nutrient Uptake: The Role of External Hyphae

During the past decade there has been a shift in mycorrhizal studies from quantification of the internal phase to assessing the external hyphal phase in soil. The external component of this symbiosis contributes to enhanced nutrient uptake of the plant primarily by extending the root's nutrient depletion zone (Sanders and Tinker, 1973). The depletion zone extends from 0.1-15 mm from the root surface, depending on the soil type and plant species (Barber, 1995). By computer modelling, Itoh and Barber (1983) determined that by doubling the length of root hairs, which have a diameter similar to some mycorrhizal hyphae, plant phosphorus (P) uptake would double. Doubling the root-P uptake rate, however, only increased P uptake by 15%. The mycorrhizal benefit is inversely proportional to the root hair length (Schweiger et al., 1992), indicating that root hairs partially offset mycorrhizal nutrient gains due to improved spatial exploitation. Various techniques have been developed to measure external hyphae (Sylvia, 1992; Dodd, 1994). As a primary tool, the use of fine-mesh screens to prevent roots from penetrating into hyphal compartments has permitted the separation of root and hyphal contribution to plant nutrient uptake (Ames et al., 1983; Schüepp et al., 1987), as well as quantification of hyphal distribution and density.

Spatial exploitation and hyphae

As reviewed previously (Bolan, 1991; O'Keefe and Sylvia, 1991), and based on plant uptake theory (Barber, 1995; Nye and Tinker, 1977), the key parameters involved

in improved nutrient uptake by mycorrhizal plants include the amount of absorbing surface area, fungal growth rates, nutrient uptake kinetics and hyphal distribution. Hyphae can extend far beyond the nutrient depletion zone (primarily P) of roots. Using an exclusion screen technique, Li et al. (1991a) located hyphae of *Glomus mosseae* up to a maximum measured distance of 11.7 cm from roots of *Trifolium repens* L. after 49 d. Ectomycorrhizal rhizomorphs are likely to extend substantially further.

In a separate comparative study on arbuscular-mycorrhizal (AM) fungi and P uptake, *Acaulospora laevis*, *Glomus* sp. and *Scutellospora calospora* developed hyphae up to 11 cm from the roots of the host plant, *Trifolium subterraneum* L., after 47 d (Fig. 1). However, hyphal densities with increasing distance from the mycorrhizal roots were not the same for all fungi. *Acaulospora laevis* had a constant hyphal density up to 11 cm, while for *Glomus* sp. it decreased after 3 cm, and for *S. calospora* the highest hyphal density was observed closest to the root and declined exponentially thereafter. The hyphal P uptake rates for the three fungi (calculated average for 28-47 d) were 2.8, 0.8 and 0.6 fmol P m^{-1} s^{-1}, respectively, with considerably higher rates for the initial 28-day period. The consequence of these differences was a substantial contrast in plant P content among the mycorrhizal treatments. The previously listed characteristics of absorbing surface area, fungal growth rates, nutrient uptake kinetics and hyphal distribution indicative of improved nutrient uptake were all favorable in the *A. laevis* treatment, which was also associated with the highest plant P content.

Depending on the mycorrhiza and the initial soil nutrient concentration, the contribution by hyphae to total plant nutrient uptake (Marschner and Dell, 1994;

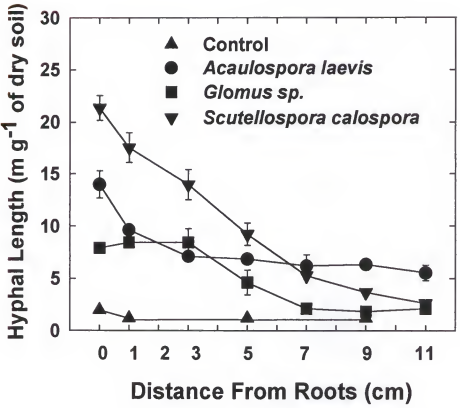

Figure 2-1. Length of external hyphae spreading from mycorrhizal roots of *Trifolium subterraneum* after (a) 28 days and (b) 47 days. Bars represent standard error of the mean [with permission from (Jakobsen et al., 1992)].

comprehensive review) has ranged between 7 to 109% for P (George et al., 1992; Li et al., 1991a; Li et al., 1991c; Pearson and Jakobsen, 1993), 16-25% for zinc (Kothari et al., 1991) and 53-62% for copper (Li et al., 1991c). In another study, in contrast to the control, mycorrhizal plants recovered 1.7 times more $^{15}NH_4^+$ applied 2 cm from the root compartment and 2.75 times more $^{15}NH_4^+$ when applied at a distance of 5 cm (Johansen et al., 1993). This provides evidence for an increasing benefit with greater distance. If diffusion and mass flow of a nutrient are slower than hyphal transport, a mycorrhizal benefit could conceivably be derived even for nutrients that are not strongly adsorbed to soils. This was demonstrated when transfer of $^{15}NO_3^-$ was increased in mycorrhizal treatments by over 400% under dry soil conditions (Tobar et al., 1994). These studies illustrate the capacity of the external phase of mycorrhizas to increase a plant's nutrient absorption. They also demonstrate that the response differs depending on the soil environment, the fungi involved and the spatial location of nutrients.

Uptake kinetics

Uptake kinetics can be quite different between mycorrhizal and nonmycorrhizal plants. Based on uptake models and mycorrhizal characteristics, however, differences in uptake kinetics appear to be of secondary importance compared to surface area and spatial distribution (O'Keefe and Sylvia, 1991). Few uptake studies comparing mycorrhizal and nonmycorrhizal plants have been performed since the review of O'Keefe and Sylvia (1991). Most studies have used root weight to standardize uptake parameters (Table 2-1). However, none have taken into account the surface area or weight

Table 2-1. Summary of nutrient uptake kinetic studies.

Fungus	Host	Nutrient concentration (μM)	K_m (μM)	I_{max}	C_{min} (μM)	Reference
Glomus fasciculatum	*Lycopersicon esculentum* (root segments)	1-100 KH_2PO_4	1.61-0.35	0.1-0.32[a]	n.d[c]	(Cress et al., 1986)
none	"	"	3.9-42	0.10-0.251[a]	n.d	
Glomus mosseae	*Glycine max* (whole plant)	30 KH_2PO_4	20	58[b]	n.d	(Karunaratne et al., 1986)
none		"	3.5	19[b]	n.d.	
Pisolithus tinctorius	*Pinus caribea* (whole plant)	20 Na_2HPO_4	3.89	0.30[a]	0.32	(Pacheco and Cambraia, 1992)
none	"	"	16.44	0.23[a]	11.98	
Glomus macrocarpon	*Zea mays* (root segments)	1.5-1070 Zn	5.3-0.38	0.08-0.47[a]	n.d.	(Sharma et al., 1992)
none	"	"	4.5-0.95	0.03-0.55[a]	n.d.	

[a] μmol g fresh weight^{-1} h^{-1}
[b] nmol m^{-2} s^{-1}
[c] n.d. = not determined

contributed by mycorrhizal hyphae, which could alter the calculated kinetic parameters. Also, in uptake experiments, the use of whole plants rather than root segments would be more appropriate, since this would incorporate possible source-sink effects of the plant. The information available suggests that there may be differences in the K_m, I_{max} and C_{min} values of mycorrhizal and nonmycorrhizal plants. The term K_m represents the substrate concentration at which the uptake rate is half of the maximum influx rate, I_{max}. The value C_{min} is the minimum solution concentration from which a nutrient can be absorbed. Studies that have estimated P uptake parameters have generally found a higher I_{max} for mycorrhizal plants. Uptake kinetics are dependent on the solution nutrient concentration (Cress et al., 1979; Sharma et al., 1992), and, consequently, selection of relevant soil nutrient concentrations in experiments is critical. For example, both P and Zn appear to have more than one concentration dependent uptake system.

Uptake kinetics may have a major role in mycorrhizal plant survival when nutrients are limited. In nature, reduced availability of nutrients occurs due to fixation and biological immobilization. With two competing plant species, the one with the lower C_{min} value would be at a competitive advantage, because it can reduce the nutrient concentration below the C_{min} value of the other organism (Tilman, 1982). In fact, Pacheco (1992) demonstrated a lower C_{min} for ectomycorrhizal compared to nonmycorrhizal pine, which suggests a potential advantage for mycorrhizal plants. Unavailable nutrients may be released in pulses when microbial activity is temporarily stimulated due to environmental conditions. Under these circumstances, plants with differing uptake strategies, such as emphasis on C_{min} in one plant versus emphasis on I_{max}

in another, could survive in the same environment due to niche separation. Mycorrhizas may add flexibility to a plant's physiological strategy by allowing it to profit from a broader range of nutrient uptake mechanisms. Furthermore, mycorrhizas may add an element of efficiency to soil nutrient exploitation by roots. Campbell et al. (1991) proposed that plant species primarily acquire resources either by efficiently exploiting a given resource (precision foraging) or by extensive development of roots and occupation of a high resource site (scale foraging). Although untested, mycorrhizas may give an advantage to the plant group with a tendency to efficiently exploit a given soil volume by accessing nutrients outside of the root's nutrient depletion zone.

Exudates and secretions

Root and hyphal secretions and exudates modify nutrient availability in the soil (Darrah, 1993; Duff et al., 1994). Several different mechanisms are involved and include release of enzymes, chelating agents such as organic anions and siderophores, and changes in rhizosphere pH by CO_2 from respiration and H^+ excretion during uptake of cations.

Nitrogen and phosphorus are often present in organic forms (Stevenson, 1986), which are less available than the organic forms. Ectomycorrhizal and ericoid fungi permit plant utilization of organic N from proteins and peptides (Abuzinadah and Read, 1986; Abuzinadah and Read, 1989; Bajwa et al., 1985; Bajwa and Read, 1985), which are otherwise unavailable N sources to plants (Abuzinadah and Read, 1986). Arbuscular-mycorrhizal fungi do not appear capable of utilizing complex organic-N sources (Frey

and Schüepp, 1993) and, thus, probably lack any significant protease release. Phosphatases are produced by plant roots (Duff et al., 1994; Tarafdar and Claassen, 1988; Cumming, 1993) and release P from bound organic forms close to the root. Phosphatase release by mycorrhizal fungal hyphae also has been demonstrated (Antibus et al., 1992; Jayachandran et al., 1992; Tarafdar and Marschner, 1994) and may increase plant uptake of P; however, mycorrhizal contribution to total P uptake by this particular mechanism has not been quantified yet. Extension of hyphae beyond the root depletion zone would permit solubilization and uptake of P from these unavailable organic-P forms. For some nonmycorrhizal plants, mobilization of the organic-P fraction can approach one third of the total P absorbed (Jungk et al., 1993). Proton release by roots, in part to compensate for NH_4^+ uptake, can create a substantially lower rhizosphere pH (Marschner and Römheld, 1983) and acidification of the soil also has been documented in mycorrhizal systems (Li et al., 1991b). Although this enhances dissolution of iron (Fe), and consequently bound P, the rate of protonation is slower than by the chelating mechanism (Schwertmann, 1991).

The availability of inorganic P bound to aluminum (Al) and Fe minerals can be increased by organic anions, such as oxalate (Fox et al., 1990), by the processes of chelation, ligand exchange and dissolution of the metallophosphate complex. Ectomycorrhiza, which form fungal mats, are capable of substantially altering the chemical soil environment by increasing the oxalate anion concentration by several orders of magnitude (Griffiths et al., 1994). Soil-solution phosphate in these mats is strongly correlated with oxalate concentration. Fungi most likely vary in the quantity of organic

acids released, resulting in differences in mineral weathering rates, nutrient release and subsequent benefit to plants. Chelation of Al not only releases bound P, but also lowers the free ion activity and thus reduces Al toxicity to the plant (Arp and Strucel, 1989). This same mechanism may apply to other metals. Chelating agents specific to Fe are termed siderophores and are produced by plant roots (Marschner, 1986), as well as by several mycorrhizal fungi (Cress et al., 1986; Schuler and Haselwandter, 1988; Watteau and Berthelin, 1995). Watteau and Berthelin (1995) found mycorrhizal siderophores of the hydroxylate type to be more effective chelators than organic anions and less specific for Fe than for Al.

The question of accessibility of organic compounds as carbon (C) sources to mycorrhizal fungi has been debated (Harley and Smith, 1983). Recently, the ectomycorrhizal fungi *Cenococcum geophilum*, *Laccaria bicolor*, *Rhizopogon vinicolor* and *Suillus lakei* were shown to utilize C from hemicellulose, cellulose and less readily from a humic polymer mix or from *Pseudotsuga menziesii* needles (Durall et al., 1994). Tanesaka et al. (1993) reported that several ectomycorrhizal fungi apparently did not have the ability to degrade complex C substances such as wood. Haselwandter et al. (1990) found several ericoid and ectomycorrhizal fungi capable of lignin degradation. At present, the ability to degrade organic matter has not been documented for AM fungi. Nonetheless, they appear to be efficient at capturing P released by decomposers prior to its being immobilized again (Joner and Jakobsen, 1994), possibly due to an advantageous spatial distribution. In general, the question of mycorrhizal fungal hyphae accessing

nutrients in forms not available to nonmycorrhizal plants may prove to be less important than the spatial accessibility of nutrients beyond the root's nutrient depletion zone.

Fungi and plants release polysaccharides resulting in a direct mucilaginous connection to soil particles in their respective rhizospheres and hyphospheres. This matrix may enhance aggregate formation, reduce nutrient loss from leaching and reduce dehydration by increasing waterholding capacity (Chenu, 1993). Furthermore, the connections to soil particles permit direct transfer of nutrients from soil to root (Uren, 1993), which may be essential under low moisture conditions, very similar to the contact exchange described by Nye and Tinker (1977).

Other Mycorrhizal Effects

Water relations

Improved water relations in mycorrhizal plants have been documented extensively (Nelsen, 1987) and have been associated with improved plant-P status (Nelsen and Safir, 1982), though non-P responses are also reported (Augé et al., 1986; Bethlenfalvay et al., 1988; Davies, Jr. et al., 1992). Drought tolerance in mycorrhizal plants is enhanced by increasing plant turgor, leaf water potential, stomatal conductance and root hydraulic conductivity. In addition, Bethlenfalvay et al. (1988) have suggested that mycorrhizal fungi are able to acquire soil water at lower water potentials than roots, although Nelsen (1987) proposed that fungal hyphae gave the plant a spatial advantage by extending the water depletion zone beyond the root. Recently, Ruiz-Lozano et al. (1995) found differences in proline concentration in drought-stressed mycorrhizal plants and suggested

that changes in osmotic potential may contribute to their improved drought tolerance over nonmycorrhizal plants. Most studies with AM fungi do not show any major water transfer via hyphae (George et al., 1992; Nelsen and Safir, 1982), although this is not always the case (Faber et al., 1991). In contrast, ectomycorrhizal fungi appear able to directly transfer water to the plant (Boyd et al., 1985), especially through rhizomorphs (Duddridge et al., 1980).

Carbon costs

The benefits of enhanced nutrient uptake associated with mycorrhizal biomass production has energy costs associated with it that vary with the symbiosis. The plant-microbe-soil interactions are unique to each environment and correspondingly the mycorrhizal response may vary (Sylvia et al., 1993). This may depend on the fungus, such as differing growth responses observed with 20 isolates of *Pisolithus* spp. on *Eucalyptus grandis* (Burgess et al., 1994). Responses also vary with plant species and cultivars of the same plant species (Krishna et al., 1985; Mårtensson and Rydberg, 1995; Smith et al., 1992). So, although host specificity *per se* has not been documented clearly, host specific responses do exist. These differences may be related to root morphology, as suggested by Baylis (1975). Negative relationships have been found between mycorrhizal dependency and root fibrousness (Hetrick et al., 1992; Pope et al., 1983) or root hair length (Crush, 1974). However, as suggested by Graham et al. (1991), other undetermined factors aside from root architecture are more likely involved. Carbon cost, measured as energy expended per unit nutrient absorbed (Tinker et al., 1994), varies

between different mycorrhizal associations. The observed variation in mycorrhizal growth response among closely related plants may relate to differing strategies of plant-C allocation to the symbiosis (Graham and Eissenstat, 1994), as well as to plant age (Eissenstat et al., 1993). Pearson and Jakobsen (1993) quantified the P-uptake efficiency (C utilized/P absorbed) for three different AM fungi. For each unit of P absorbed they found that *Scutellospora calospora* and a *Glomus* sp. utilized 25 and 16 times more C, respectively, than *Glomus caledonium*. Total-C partitioning belowground was higher in the less efficient mycorrhizal fungi, indicating that energy efficiency of the symbiosis may be one reason for differing plant growth responses to fungi.

When comparing carbon costs of mycorrhizal to nonmycorrhizal plants, 4-36% more of the total C fixed is allocated belowground due to mycorrhizas (Durall et al., 1994). To distinguish nutritional from other mycorrhizal effects on plant-C balance, mycorrhizal plants were grown at high soil-P concentrations and demonstrated a 37% higher belowground carbon allocation than nonmycorrhizal plants (Peng et al., 1993). Of this 37%, 51% was attributed to greater root biomass and 10% to construction costs of lipid-rich roots most likely associated with the mycorrhizal fungus. Enhanced photosynthesis in mycorrhizal plants can compensate to varying degrees for this increased C drain (Dosskey et al., 1990; Kucey and Paul, 1982). Plant root turnover is also associated with a high C cost, although few studies have assessed the role of mycorrhizas in controlling this process. Durall et al. (1994) determined that ectomycorrhizal roots have a lower root turnover rate than nonmycorrhizal roots. In environmental conditions where nutrient pulses occur, roots with a lower root turnover rate demonstrated a

competitive advantage (Campbell and Grime, 1989). This suggests that mycorrhizal plants may profit from the reduced root turnover rate by having to invest less C into nutrient absorbing structures.

Plant fitness

Although many of the previous topics dealt with improving plant growth and stress adaptation, few mycorrhizal studies have directly addressed mycorrhizal influence on plant fitness, that is the plant's ability to increase its numbers proportionately to other species (Begon et al., 1986). Enhanced efficiency of resource acquisition by mycorrhizal plants allows more energy to be allocated to growth and reproduction, which potentially increases plant fitness. The result in the next generation may be expressed in terms of improved survival, growth rate or reproduction. Mycorrhizal plants have displayed increased seed number, seed weight and P and N content (Lu and Koide, 1994), with some benefits still significantly expressed in the second generation of offspring grown in the absence of mycorrhizal fungi (Koide, T. and Lu, 1992). Increased P status of seed has been associated with subsequently higher P content and plant biomass (Bolland and Paynter, 1992). For some plant species, the presence of mycorrhizas enhanced seedling emergence rate (Hartnett et al., 1994). High P concentration in seed has resulted in increased number of emerging seedlings and a higher rate of emergence (Thomson and Bolger, 1993), factors which also have been identified as important predictors of competitive success in secondary succession (Stockey and Hunt, 1994).

Xenobiotics

In many ecosystems plants and mycorrhizal fungi are exposed to a wide variety of toxic compounds (xenobiotics and in some instances naturally occurring toxic compounds). Mycorrhizal fungi may effectively mediate and alter the interaction between plant and xenobiotic compounds. Various papers have assessed or reviewed pesticide effects on mycorrhizal fungi (Dehn et al., 1990; Trappe et al., 1984). Mycorrhizal fungi may function in the translocation of herbicides. In one study with apple and three herbicides (dichlobenil, paraquat and simazine), root dry weight of noninoculated plants exposed to herbicides was reduced by 46% in contrast to a 63% decrease in mycorrhizal plants (Hamel et al., 1994). Although no effect on hyphal length was found at the highest simazine concentration applied, 75% of the mycorrhizal plants died compared to none in the control treatment. The authors attributed this to facilitated herbicide flow to the host plant mediated by the mycorrhizal fungus. Uptake and translocation of the herbicide atrazine was also found in mycorrhizal corn, which is atrazine-tolerant (Nelson and Khan, 1992). Although the quantity absorbed was small compared to direct root uptake, the question of how this may affect an atrazine-sensitive plant remains unanswered. Certain mycorrhizal fungi also have demonstrated the capacity to degrade herbicides such as atrazine and to a lesser extent 2,4-dichlorophenoxyacetic acid (Donnelly et al., 1993). This provokes the question as to whether mycorrhizal fungi offer some protection against xenobiotics. In corn and sorghum certain herbicide safening effects by AM fungi have been found against the herbicides imazaquin, imazethapyr and pendimethalin (Siqueira et al., 1991).

Metal cations and soil acidity

High metal cation concentrations can be toxic to plants. The high solubility of Al, due to the acidic nature of Oxisols and Ultisols, is a growth-limiting factor for plants in many tropical countries. Natural selection of mycorrhizal ecotypes leads to varying genotypic sensitivity to soil acidity (Robson and Abbott, 1989), as well as to high metal concentrations (Gildon and Tinker, 1983; Griffioen et al., 1994). Several studies have found mycorrhizas capable of alleviating toxic effects to plants caused by Al, cadmium, Cu and Zn (Bradley et al., 1982; Colpaert and Van Assche, 1993; Denny and Wilkins, 1987; Dueck et al., 1986; Koslowsky and Boerner, 1989). Two mechanisms currently explain this response. Firstly, electronegative sites on the hyphal cell walls bind the positively charged heavy metal cations (Denny and Wilkins, 1987; Galli et al., 1994). The observation that under acidic soil conditions heavy metal uptake is increased (Killham and Firestone, 1983) partly confirms this. It is possible that protonation of negatively charged sites in the plant or fungal walls results in less binding and greater uptake of the metal cation. The second path is the immobilization of the cations by complexation in vacuoles with polyphosphates (Martin et al., 1994) or associated metallothionein-like peptides (Turnau et al., 1994).

Synecology

Co-evolution of mycorrhizal fungi and plants has been suggested (Allen, 1991; Harley and Smith, 1983). Since selection for more fit species occurs continuously, and a larger proportion of plants show mycorrhizal dependency than not, it follows that there

must be some measure of improved fitness derived from mycorrhiza; otherwise the symbiosis would have been selected against. The alternative is that mycorrhizal fungi are parasites with maximum adaptability to plant resistance strategies. This, however, is unlikely considering the exchange of nutrients between the two organisms, which is characteristic of a true mutualism.

The effects of the mutualism on plant growth and survival influence interactions beyond the single plant level (Brundrett, 1991; Francis and Read, 1994). Plants rarely grow alone, except in extreme or anthropogenic environments, and consequently end up competing for similar resources, especially inorganic nutrients, water and light. Under conditions limiting growth, mycorrhizal plants have distinct competitive advantages. Thus, from a holistic and functional perspective, mycorrhizal research reaches its full value when applied to natural or managed ecosystems where interactions occur. Current issues pertain to the involvement of mycorrhizas in plant community development, stabilization and diversity, as well as to questions of environmental sustainability and the economics of agricultural production systems. A relevant question, then, is to what extent is the force of this symbiosis manifested in plant communities?

Plant Interactions

During competition, plants utilize several different strategies for optimal resource capture with many of them overlapping those found in the mycorrhizal symbiosis. The choice of strategy depends primarily on a site's resources and the amount of disturbance (Grime, 1979; Tilman, 1982). Literature summarized in the first part of this chapter has

shown that mycorrhizas can enhance resource capture. Environmental factors strongly influence the mycorrhizal benefit derived by a plant and consequently also its competitive ability.

Resource competition

Competition occurs when a resource is inadequate to meet the needs of the competitors. Nutrient availability fluctuates with the chemical environment and moisture content of the soil. Soil heterogeneity frequently compounds the intensity of competition in some areas, since resources are not evenly distributed. Phosphorus has been the focus of mycorrhizal research, because it is required by plants in proportionately large quantities, and yet, in the soil it is easily immobilized chemically and biologically. Consequently, the use of P also dominates mycorrhizal studies involving competition.

When mycorrhizal plants compete under nutrient-limiting conditions, niche differentiation may be of considerable importance. Plants competing intraspecifically will have similar nutrient requirements and acquisition strategies which may vary depending on plant age. Conversely, in interspecific interactions, some competition may be alleviated by niche differentiation. For example, a potential growth response associated with spatial niche separation by roots of two grass species only became evident by experimentally increasing soil depth (Van Auken et al., 1994). The varying plant responses to different mycorrhizal species in the literature suggest the involvement of a combination of the earlier reviewed mechanisms, including hyphal spatial distribution and access to less available nutrients. However, if the mycorrhizal contribution to nutrient

uptake is primarily related to spatial niche differences between roots and hyphae, then larger soil volumes would be preferable in experiments; otherwise root nutrient depletion zones quickly overlap and the potential mycorrhizal benefit is not realized (O'Keefe and Sylvia, 1991). As an intermediate approach between pot and field competition studies, artificial micro- or mesocosms have been used (Campbell et al., 1991; Grime et al., 1987), which, among other things, allow for the exploration of large soil volumes by external hyphae, the creation of resource gradients or patches and the longer-term monitoring of plant growth and reproduction in a regulated environment. Further consideration should be given to the incorporation of an unsterilized soil control into experiments. The inclusion of plant pathogens, soil arthropods and microbes which affect resource abundance and mycorrhizal plant growth (Newsham et al., 1994), as well as subsequent plant interactions, would provide a more realistic extrapolation of experimental results to natural phenomena.

A number of mycorrhizal plant competition studies have demonstrated that AM fungi affect competition to varying degrees (Brown et al., 1992; Francis and Read, 1994; Fitter, 1977; Hetrick et al., 1989; Hartnett et al., 1993; Newman et al., 1992). Plant competition between two host plants involving a single species of AM fungus account for the majority of the data. Apparently only one plant competition study dealt with different groups or species of mycorrhizal fungi and it is also the only EM plant competition study (Perry et al., 1989). There is evidence that competitive success is related to mycorrhizal dependency (Hartnett et al., 1993; Hetrick et al., 1989). Mycorrhizal dependency is very variable and depends on the particular environment and host plant. Hartnett et al. (1993)

and Bååth and Hayman (1984) determined that, in a given soil volume, mycorrhizal benefit for a plant decreases with increasing density of its competitors. Higher plant density is paralleled by an increase in root and hyphal density in the soil and proportionately greater overlap of nutrient depletion zones. In intraspecific competition of inoculated plants of high mycorrhizal dependency, density-related competition was observed, but this did not occur when mycorrhizal fungi were absent. Inoculated plants with low mycorrhizal dependency lacked this response, indicating their ability to more efficiently extract nutrients from the soil than the nonmycorrhizal plants with high mycorrhizal dependency.

Plants of the same species but different plant age also have been compared for competitive interactions. Eissenstat and Newman (1990) evaluated the possible advantages of mycorrhizas to seedling establishment in the presence of an older plant of the same species. The results indicated that there is not a facilitative but rather a competitive relationship between the two plants, similar to that observed in the absence of mycorrhizal fungi. In another study, Franson et al. (1994) found that competition intensity between an established and a seedling soybean plant was not altered by increasing the stress on the younger plant.

Plant competitive interactions between mycorrhizal host and nonhost plants have been investigated in a limited number of studies. It is worth noting that some have documented a reduction in biomass of nonhost plants when such plants were grown under mycorrhizal conditions (Allen et al., 1989; Ocampo, 1986). Francis and Read (1994) found evidence for a chemical factor, which was extracted from soil of mycorrhizal

plants, that inhibited root growth of nonhost plants. This suggests that mycorrhizas may have effects beyond those currently known.

In summary, mycorrhizas can enhance a plant's competitive ability, and the effect is generally associated with increased nutrient uptake. The greatest benefit of mycorrhizas appears to lie in their ability to buffer the plant from adverse environmental conditions that reduce resource availability.

Mycorrhiza-mediated reduction of competition

With most plants possessing similar nutritional requirements, competition is a key factor in their interactions. The existence of hyphal connections between plants is well known. Various studies, especially those using root-excluding screens, have unequivocally demonstrated that nutrient transfer between root zones of a donor and receiver plant can be mediated by mycorrhizal hyphae (Newman, 1988; Newman et al., 1992). Although it is possible for hyphae from the receiver mycorrhiza to scavenge nutrients from the rhizosphere of the donor plant, most likely the majority of transfer is by direct hyphal connections between plants. For example, radio-labelled C from an ectomycorrhizal donor plant has been found solely in ectomycorrhizal plants and not in neighbor AM neighbor plants; by using autoradiography, no visual evidence existed of a direct interspecific C transfer between intermingling roots of donor and receiver plants (Read et al., 1985). In another study, 46% of the total C transferred directly from plant to plant was via mycorrhizal connections, 15% of uptake was indirectly mediated by mycorrhiza, and 39% was translocated by other processes (Martins, 1993). These

fractions could be verified further by comparing nutrient transfer from a mycorrhizal donor plant to either a myc$^-$ mutant (a mutant plant not able to form mycorrhiza) or a normal mycorrhizal receiver plant. The quantity obtained by the receiver is variable, and appears to depend on the nutrient involved. Generally, P is not transferred at fast rates (Newman and Eason, 1993) or in quantities that significantly affect growth (Ikram et al., 1994). The transfer of N by mycorrhizas has been documented (Newman, 1988), with most studies utilizing a legume, because of its importance in intercropping systems, as the donor plant. The quantity of N transferred from the root zone of donor plant to the receiver plant varies (Bethlenfalvay et al., 1991; Frey and Schüepp, 1993). By increasing competitive pressures for N in intercropping systems, mycorrhizal fungi at certain times may enhance nitrogen fixation (Barea et al., 1989), although this is not always the case (Reeves, 1992). Both of these studies and others (Hamel and Smith, 1991; Ikram et al., 1994) have found minimal amounts to no N transferred. The quantitative significance of mycorrhizal transfer of nutrients to total uptake by the receiver plant still remains unclear.

The phenomenon of increased survival of certain plant species in mycorrhizal microcosm studies (Grime et al., 1987) deserves further attention, especially, since no direct cause was found. Source-sink gradients, such as those created by shading or low-nutrient status of one plant, have been suggested as the force behind nutrient transfer. For interplant C transfer, shading of the receiver plant increased C translocation to that plant (Read et al., 1985). However, shading does not always produce this effect (Franson et al., 1994; Hirrel and Gerdemann, 1979). In contrast, clipping of leaves to simulate

herbivory and to produce a C sink resulted in C transfer away from the clipped plant (Waters and Borowicz, 1994). In settings where young seedlings compete for nutrients with established plants, the seedlings become more quickly colonized by the preexisting mycorrhizal network; however, no further benefit to the seedlings was detected (Franson et al., 1994; Eissenstat and Newman, 1990). In Grime's (1987) study, ^{14}C-labelling of one dominant plant resulted in substantially more C being transferred to subdominants when plants were mycorrhizal compared to nonmycorrhizal. Although competition does occur in these systems, several plants colonized by the same mycorrhizal type will be closely tied together by the hyphal network and may benefit from C transfer among plants.

Environmental Conditions and Plant Competition

Non-resource edaphic factors

Several soil characteristics may indirectly influence the assorted mycorrhizal mechanisms that enhance a plant's competitive ability. Soil acidity is an important factor influencing soil nutrient availability. Acidic soils are a natural result of soil weathering, and, as stated earlier, Al toxicity is one of the main associated problems. Mycorrhizas may enable a plant to survive unfavorable conditions caused by toxic concentrations of metal cations, including Al (Koslowsky and Boerner, 1989). Although mycorrhizas may facilitate growth of plants under acidic soil conditions, I am not aware of any studies that systematically address the effect this may have on plant competition.

Soil chemical processes associated with organic matter turnover and mycorrhizas may also play a yet unstudied role in plant interactions. As organic matter is degraded by microbes, various compounds, including phenolic materials, are released to the soil. Phenolics have been implicated in various allelopathic interactions (Rice, 1984). Researchers have demonstrated both inhibition and stimulation of mycorrhizal fungi by phenolic compounds (Baar et al., 1994; Boufalis and Pellissier, 1994; Siqueira et al., 1991). Different microbial responses to phenolics have been attributed to variability in degradation capacity of the microbes, phenolic concentration, soil characteristics and availability of inorganic soil nutrients (Blum and Shafer, 1988). Similarly, mycorrhizal fungi vary in their capacity to chemically alter different forms of phenolic compounds (Giltrap, 1982; Ramstedt and Soderhall, 1983; Tam and Griffiths, 1993). Garbaye (1994) hypothesized that phenolic compounds may be degraded by bacteria closely associated with mycorrhizal fungi, thereby also enhancing the establishment of mycorrhizal fungi. Although no clear link has been found between mycorrhizal sensitivity to phenolic compounds and plant competitive ability, the results of a few studies suggest a possible connection (Leake et al., 1989; Wacker and Safir, 1990). Because mycorrhizal fungi occur in competitive environments, such as forests, with the potential of allelopathy (Horsley, 1987; Pellissier, 1994), it is important to determine what growth-limiting factors, as well as their magnitudes, actually occur. Although it is difficult to distinguish resource competition from interference competition, several researchers have been successful in differentiating these two phenomena (Nilsson, 1994; Shilling et al., 1992; Thus, 1994; Wardle et al., 1994).

Associated soil biota

Fitter and Garbaye (1994) have summarized the current information about belowground interactions of mycorrhizas and rhizosphere microbes. Unfortunately, few studies have addressed how these interactions affect plant populations or communities. Mycorrhizas influence the rhizosphere environment by modifying plant exudation and rhizodeposition (Leyval and Berthelin, 1993), and subsequently affect microbial composition and metabolic activity in varying degrees. Inversely, certain fluorescent pseudomonads and spore-forming bacilli, similar to growth-promoting rhizobacteria, may significantly regulate the mycorrhizal benefit to the plant (Garbaye, 1994; Schreiner and Koide, 1993); however, mechanisms of action are still largely unknown. These bacteria have demonstrated some fungal, but not plant, host specificity. With appropriately matched mycorrhizal fungi and bacteria it is conceivable that a plant would possess a competitive advantage over other plants without selected associations. Rabatin and Stinner (1991) reviewed the effects of microfauna, many of which are fungivores, on mycorrhiza. As an example, Boerner and Harris (1988) conducted a competition study between mycorrhizal *Panicum virgatum* and the nonhost *Brassica napa*, where the addition of Collembola reduced the competitive ability of the grass, resulting in a reduction of biomass compared to the mycorrhizal *P. virgatum* without competition.

Studies of plant disease control by mycorrhizas interacting with plant pathogens have yielded variable results (Linderman, 1994; Duchesne, 1994). Various mechanisms have been reported that are unique to the environment, host and microbes involved. Based on field studies utilizing the fungicide benomyl, Carey et al. (1992) suggested that,

aside from direct physiological benefits to the plant, mycorrhizal contributions to plant health in the field may be a common but subtle phenomenon, because it is buried within complex interactions.

To make the situation more complex, few studies have included interactions between mycorrhizas and other plant endophytes (Clay, 1992). The fungal endophyte *Acremonium* sp., for example, has reduced colonization and reproduction by *Glomus* sp. (Chu-Chou et al., 1992; Guo et al., 1992). Reduction of insect herbivory has been attributed to secondary metabolite production by fungal endophytes (Clay, 1991). Another study found that mycorrhizas may reduce feeding inhibition of an insect herbivore induced by *Acremonium* sp. (Barker, 1987). Additionally, nonmycorrhizal endophytes are capable of altering competitive relationships between plants (Clay et al., 1993) and plant drought resistance (White, 1992) in ways similar to mycorrhiza. The data suggest that endophytes are involved in various effects observed in plant studies and consequently they deserve further consideration.

Herbivory

Herbivores generally have either an inhibitory or neutral effect on mycorrhizas (Barbosa et al., 1991; Gehring and Whitham, 1994). Herbivory results in increased plant-C allocation to the replacement of aboveground parts instead of to maintenance of the mycorrhizal symbiosis (Jones and Last, 1991). There are also a few studies on the inverse effect of mycorrhizas on herbivores (Gange et al., 1994; Rabin and Pacovsky, 1985). Generally, mycorrhizas had an inhibitory effect on the herbivorous insects. Gange

and West (1994) found that compared to fungicide-treated plants, mycorrhizal plants had lower soluble neutral sugars, starch, total N, and amino acids (alanine and tyrosine/valine) and a higher concentration of the anti-feedant chemicals, aucubin and catalpol. In their study, chewing insects were negatively impacted when feeding on mycorrhizal plants; however, sucking insects developed better on mycorrhizal plants. The authors hypothesized that a higher C/N ratio in the mycorrhizal plants allowed more C to be allocated to plant defense mechanisms, such as secondary plant metabolite production. Localization of the secondary metabolites may partly account for the differential response between insect types. Viewed in terms of plant competition, plants able to efficiently modify their C balance to simultaneously reduce insect pests and still maintain the mycorrhizal association may have a competitive advantage in the long run.

Plant Succession and Community Structure

Limited information is available on the ecological relevance of mycorrhizas in plant competition. Plant competition can be viewed in terms of single plant interactions, but its importance lies at the population and community levels. The interactions occurring at the ecosystem level are obviously complex and many have been set aside for the sake of simplicity. As has been suggested by various authors (Brundrett, 1991; Francis and Read, 1994; Newman, 1988) mycorrhizas are likely involved in plant community structuring, but the magnitude of their effect is unknown. Increasing the competitive ability of individuals within a population enhances their ability to capture resources and improves their fitness. One of the major components determining early succession is

plant competition for limited nutrients (Wilson and Shure, 1993). Under nutrient limitations, resource acquisition enhanced by mycorrhizas occurs at the expense of other plants, which results in the highly competitive plants becoming more abundant and dominant in the community. Continuous growth of a plant in the same soil eventually will select a microbial community well adapted to that environment. Over time the adapted microbial community can become disadvantageous for growth of that plant species, but not for others, and, in this manner, may contribute to plant succession (Bever, 1994; Van der Putten et al., 1993). In these studies it was suggested that this negative feedback on growth may be related to pathogen buildup. Mycorrhizal fungi were not considered, because of the assumption that mycorrhizal effects are usually beneficial. However, if there is a selection for less efficient mycorrhizal fungi occurring over time, then this may similarly contribute to succession by decreasing a plant's C-use efficiency and its competitive ability. In monocultural settings, a shift of mycorrhizal fungal species composition over time was identified by Johnson et al. (1992a; 1992b) and Wacker et al. (1990). In both cases there was an associated decline in plant growth, indicating that mycorrhizal fungi should not be discarded *a priori* as a contributing factor to growth declines.

Succession of ectomycorrhizal fungi from "early" to "late" stage fungi occurs in undisturbed forest systems (Deacon and Fleming, 1992). Differing fungal resource requirements, as well as changes in other soil microbial components, have been postulated to cause the succession (Garbaye, 1994). Recent research indicates that this succession may be tied closely to factors found in the soil organic matter. Removal of

litter and humus in *Pinus sylvestris* stands increased mycorrhizal fungal species richness and reverted the species composition to the early successional types (Devries et al., 1995). In other systems, the increased buildup of organic matter also has been associated with higher concentrations of phenolic compounds (Kuiters and Sarink, 1986; Leake et al., 1989), which have demonstrated allelochemical effects. Perhaps resistance to and the ability to degrade phenolic compounds determines which fungal species are capable of growing at a certain stage of succession. Leake et al. (1989) demonstrated that ericoid mycorrhizas were capable of enhancing ericoid plant growth and survival, possibly by a detoxification mechanism. Whereas AM fungi are found more commonly in mineral soils, ectomycorrhizal fungi are often associated with environments high in organic matter and are physiologically adapted to utilizing complex substrates (Francis and Read, 1994). Also, ectomycorrhizal mantles surrounding root tips are capable of protecting these from potentially toxic compounds. As a consequence, tolerance to adverse environmental conditions allows the plant to focus more of its energy on resource acquisition strategies without substantial tradeoffs of energy for other mechanisms, thereby making it a better competitor.

Plant competition, as affected by mycorrhizal fungi, could be relevant in plant community structuring and succession. As such, mycorrhizal benefits to single plants may prove functionally significant at the ecosystem level. In addition, positive interactions in communities are often neglected (Bertness and Callaway, 1994) and should also be considered in the discussion of plant interactions mediated by mycorrhizas (Amaranthus and Perry, 1994). Mycorrhizas can moderate plant competition (Perry et al., 1989) and

provide resilience to disturbance (Amaranthus and Perry, 1994). Mycorrhizal connections between dying and living plants also limit soil nutrient loss by leaching and immobilization (Eason and Newman, 1990). The network of hyphal bridges connecting neighboring plants can affect coexistence by increasing species richness and diversity (Gange et al., 1993; Grime et al., 1987). The current literature indicates that this is perhaps more likely due to transfer of C than of inorganic nutrients. Furthermore, a higher plant species diversity has been associated with increased ecosystem stability in a stressed environment (Tilman and Downing, 1994). Obviously, with the multitude of effects and interactions mediated by mycorrhiza, a quantification of the net mycorrhizal influence in ecosystems is a formidable challenge. Still, with the current emphasis on environmentally sound management of ecosystems, it is important to include them in considerations of appropriate technologies in managed ecosystems.

CHAPTER 3
LIMITATIONS IN THE USE OF BENOMYL IN EVALUATING MYCORRHIZAL FUNCTIONING

Introduction

A limitation to mycorrhizal field research is the difficulty of obtaining an appropriate nonmycorrhizal control, since plants in nature are normally colonized. Soil fumigation has been used to control mycorrhizal fungi; however, the broad biocidal effects limit the usefulness of this technique. Fungicides are more specific and alter fewer biological soil processes. Paul et al. (1989) summarized the ideal properties of a fungicide used to chemically exclude an organism from an experiment. The fungicide properties should include: (i) moderate persistence to reduce mechanical disturbance from the application process, (ii) an appropriate activity spectrum that targets selected organisms only and (iii) no direct physiological effects on the plant.

The systemic fungicide benomyl, a benzimidazole, has been used frequently to reduce arbuscular mycorrhizal (AM) activity in experimental treatments (Jalali and Domsch, 1975; Kough et al., 1987; Fitter and Nichols, 1988; Hartnett et al., 1994; Newsham et al., 1995; West et al., 1993). Benomyl's lack of direct effects on plants and somewhat selective effects against AM fungi (Zygomycetes) currently make it a better choice compared to other fungicides (Paul et al., 1989; Sukarno et al., 1993). Nonetheless, the amount of mycorrhizal control achieved with benomyl has varied. Reduction of colonization or biomass of mycorrhizal plants has been observed in several

cases (Evans and Miller, 1988; Fitter and Nichols, 1988; Trappe et al., 1984), but these results are not always achieved (Koide et al., 1988; Fitter, 1986; Trappe et al., 1984). Much of this variability is likely attributable to the experimental conditions such as soil type, method and timing of fungicide application and potentially more complex interactions occurring within the soil microbial community. For example, benomyl can inhibit nematodes (Elamayem et al., 1978) and different fungi that do not form mycorrhizas (Edgington et al., 1971), thereby indirectly altering mycorrhizal effects.

Several studies have addressed the effects of arbuscular mycorrhizas on plant interactions (Fitter, 1977; Hall, 1978; Newman et al., 1992), and some have utilized benomyl (Hartnett et al., 1993; Hetrick et al., 1989; Newsham et al., 1995) or other fungicides (Gange et al., 1993) to create control treatments. Only one study addressed the influence of EM fungi on plant competition (Perry et al., 1989). Very few studies have taken place under field conditions, and apparently none have addressed the role of mycorrhizas in the interactions between AM and EM plants. Benomyl's putative selective effect against AM fungi and neutral effects on EM fungi (Trappe et al., 1984) could be valuable in sorting out the individual benefits of these two types of mycorrhizal symbioses to different host plants competing for the same nutrients.

As part of a larger plant competition study between AM and EM plants, the usefulness of benomyl as a tool to selectively control mycorrhizas was tested. The main objectives were to: (i) compare the efficacy of benomyl in controlling mycorrhizas in the greenhouse to that in the field, (ii) differentiate effects of benomyl on external hyphae

from those on the internal mycorrhizal phase and (iii) determine if the intensity and longevity of the fungicide's effect was dose-dependent.

Materials and Methods

Field Study

The site was located 21 km northwest of Gainesville, Florida and was part of a larger plant competition study involving slash pine (*Pinus elliottii* Engelm. var. *elliottii*)) and weeds. Slash pine had been planted in April 1990 in beds approximately 26 cm in height and about 2 m in width with rows spaced approximately 213 cm apart. Soil was a Pomona fine sand (a sandy, siliceous, hyperthermic Ultic Haplaquod). The surface 10 cm of soil had 7 μg P g^{-1} extractable in 2 mM $CaCl_2$ and a soil solution with pH 3.9. Approximately 3.3% weight was lost upon ignition. The dominant weeds were *Panicum chamaelonche* Trin., *P. aciculare* Dec.ex Poir. in Lam., *Andropogon* spp., *Paspalum* spp. *Rubus* sp. and *Serenoa repens*. In December 1991, less than 1 spore of mycorrhizal fungi g^{-1} of field soil was detected; the populations consisted of a mix of *Glomus* sp., *Gigaspora* sp. and *Scutellospora* sp. In the greenhouse, pot cultures of *P. chamaelonche* originating from the field and grown in field soil yielded two AM isolates, *Gigaspora rosea* (INVAM FL224) and *Scutellospora heterogama* (INVAM FL225) which were submitted to and identified by J. Morton at INVAM.

Two areas (each 18.4 m by 11 m) containing slash pine and weeds were selected randomly for this study. The control plot received no fungicide sprays. Benlate® 50 DF (E.I. du Pont de Nemours & Co., Inc., Wilmington, DE) was applied to the second area

with a CO_2-pressurized backpack sprayer by covering the area once and then making a second application perpendicular to the first. The first spray (2 April 1991) was applied at the rate of 5 kg benomyl ha^{-1} using the equivalent of approximately 150 ml of water m^{-2}. Subsequent sprays (30 May, 11 July and 19 Sept. 1991) were applied at a rate of 20 kg benomyl ha^{-1}.

Panicum chamaelonche was chosen as the indicator plant of AM fungal activity because it was a dominant weed species at the site. Samples were taken on 2 April, 4 April, 30 May, 10 June, 2 July, 22 July, 13 August, 10 October 1991. At each sampling, three plants were selected randomly and removed from each plot. The roots were washed and cut into lengths of 1 to 2 cm. To determine fungicide effects on colonization and metabolic activity, 1- to 2-g subsamples of roots were stained at room temperature for 8 h in a solution containing 0.2 M Tris HCl (pH 7.4), 1 mg ml^{-1} of iodonitrotetrazolium violet (INT) and 3 mg ml^{-1} of NADH (Sylvia, 1988). This was followed by clearing the roots in a boiling, saturated solution of chloral hydrate for 10 min and subsequent counterstaining overnight in 0.5% aniline blue in lactoglycerol. The chloral hydrate treatment proved unnecessary and was eliminated in samplings collected after May. The roots were destained in lactoglycerol and a minimum of 25 1-cm-long root segments per plant were laid out parallel to each other on slides. The percentage of root segments with arbuscules and the percentage of total arbuscules that were active (those staining with INT) were estimated using bright-field microscopy at 400x magnification. The effect of benomyl on mycorrhizal development was evaluated using the relationship of time and either arbuscule abundance or activity. The slopes of linear regression of benomyl-treated

versus nontreated plants were compared using the General Linear Model procedure of SAS (SAS Institute, Inc., 1989).

Greenhouse Study

Both of the following experiments had completely randomized factorial designs (two mycorrhizal treatments x four benomyl levels) with seven replications each. To maintain uniform daylength of approximately 12 h, extra light (800 μmol m^{-2} s^{-1} from 17:00 to 20:00 hr) was provided. Plants in all experiments were fertilized semiweekly with 3.2 μM NH_4NO_3, 7.5 μM $Ca(NO_3)_2$, 7.7 μM KCl, 1.0 μM $MgSO_4$, 20 nM NaFeEDTA, 5.0 nM $CuSO_4 \cdot 4H_2O$, 240 nM H_3BO_4, 20 nM $MnCl_2 \cdot 4H_2O$, 5 nM $Na_2MoO_4 \cdot 2H_2O$ and 20 nM $ZnSO_4 \cdot 7H_2O$. The nutrient solution for corn or pine contained, respectively, 3.2 nM H_3PO_4 or 0.32 nM H_3PO_4. All data were analyzed by analysis of variance using the General Linear Model procedure (SAS Institute, Inc., 1989). Both experiments were repeated once under similar environmental conditions.

Benomyl effects on pine

Slash pine seeds were disinfested for 2 min in a 5.25% sodium hypochlorite solution with 0.2 ml Liqui-Nox surfactant (Alconox, Inc., New York, NY) and then rinsed thoroughly with tap water. Plants were raised from seed for 12 d in a growth chamber [29 C°/23 C° (day/night), with a 15-h light period and irradiance of 1000 μmol m^{-1} s^{-1}] in a vermiculite/sand (1:1) mix. They were then transplanted into sand in 50-ml pots (5 cm^2 of surface area) grown in the greenhouse for 6 wk where they received water

only. *Pisolithus tinctorius* (Pers.) Coker & Couch (isolate S106) was grown with no shaking in a modified Melin-Norkrans liquid medium (Marx, 1969) containing glucose instead of sucrose. Just prior to use, fungal mats were washed with tap water, added to a food processor with water and chopped (Rousseau and Reid, 1990). Eight-week-old pines were inoculated with the fungus by immersing the washed roots in the suspension and then grown in the greenhouse in 500 ml of sand in Deepots™ (28 cm^2 of surface area; McConkey, Co., Sumner, WA). Six weeks after inoculation, 10 ml of a suspension of Benlate® 50 WP in deionized water was applied once at 0, 20, 60 or 150 kg benomyl ha^{-1} equivalent (based on pot surface area). Plants were grown from January to March 1993 under a mean photosynthetic photon flux density of 535 μmol m^{-2} s^{-1} and 17/30°C (min./max.) temperature regime.

Groups of plants were harvested before, and then 2 and 4 wk after benomyl application. Prior to harvesting the plants, a soil core (15.5-mm diam. by 15-cm deep) was removed from each pot. Hyphal length and activity were evaluated by a slightly modified procedure of Sylvia (1988). A thoroughly mixed, 10-g, wet-mass subsample of soil was added to 500 ml of water and chopped in a Waring blender at the high setting for 20 seconds. The resulting suspension was allowed to settle for 20 seconds before a 25-ml portion was removed and filtered through a 0.45-μm-pore size membrane (GN-6 Metricel™; Gelman, Ann Arbor, MI). The hyphae on the membrane were stained for 6 h with INT solution, destained with tap water, counterstained for 30 min with 0.1% trypan blue in lactoglycerol and destained again with tap water. Using a gridline-intercept

method, total and active hyphal lengths were determined microscopically at 400x from 20 randomly selected fields on the filter.

Pine needles were removed from seedlings and dried overnight at 65°C, and P content was determined colorimetrically (Murphy and Riley, 1962). Ergosterol (a sterol found in fungal, but not plant, membranes) content in the root was used to provide a relative estimate of total fungal biomass present (Martin et al., 1990; Salmanowicz et al., 1989). Fresh roots were washed, ground in liquid nitrogen and thoroughly mixed. A 0.1- to 0.3-g subsample was extracted overnight at room temperature with 5 ml of 100% ethanol. This sample was filtered through a 0.45-μm syringe filter and then assayed for free ergosterol by high-pressure liquid chromatography (Waters 715 Ultra WISP, Gilson 115 UV detector). Separation was achieved on a C-18 column (Supelcosil™ LC-18; Supelco, Inc., Bellefonte, PA) at 40°C using a methanol-water mobile phase (92:8) flowing at 2 ml min^{-1} with detection at 282 nm.

Benomyl effects on corn

The effect of benomyl on colonization by the AM fungus *Glomus* sp. (INVAM FL329, formerly FL906) was studied in a separate experiment. Germinated corn (*Zea mays* L. cv. Silver Queen) seed was planted in sand in Deepots™ with 5 g of soil inoculum (83 spores g^{-1}) placed 2 to 3 cm below the seedling. Control plants received a 5-ml suspension of inoculum filtrate obtained by mixing 60 g of soil from a pot culture with 1.2 L of water and then filtering this through a 10-μm membrane filter. Benlate 50 WP was applied 19 d after planting to the soil surface at rates of 0, 20, 60 and 150 kg

benomyl ha^{-1} equivalent. Plants were grown from March to May 1993 under a mean photosynthetic photon flux density of 608 μmol m^{-2} s^{-1} and 18/35°C (min./max.) temperature regime.

The plants were sampled before, and then 2, 4 and 6 wk after benomyl application. The harvest procedures were the same as for pine, with the exception of estimation of root colonization. Washed root segments (1 to 2 cm) were cleared with 10% KOH for 30 min, rinsed several times with tap water, acidified for 30 min in 5% HCl and stained overnight in 0.05% aniline blue in lactoglycerol. Colonization was determined using a gridline-intersect method (Giovannetti and Mosse, 1980). Although fungi other than AM existed in this particular system, the differentiation of saprophytic from characteristic AM fungal hyphae was based on gross morphological differences. Arbuscular mycorrhizal fungi generally had a somewhat larger hyphal diameter (4 compared to <2 μm), stained darker with aniline blue, were not dematiacious, lacked septation or clamp connections and demonstrated a less angular growth pattern compared to other fungi present. Prior to statistical analysis, percentage colonization was transformed using the arcsine, square root transformation.

Results

Field Study

Initial AM colonization of *P. chamaelonche* in the field was high, indicating that root growth and mycorrhizal development commenced earlier than the first fungicide

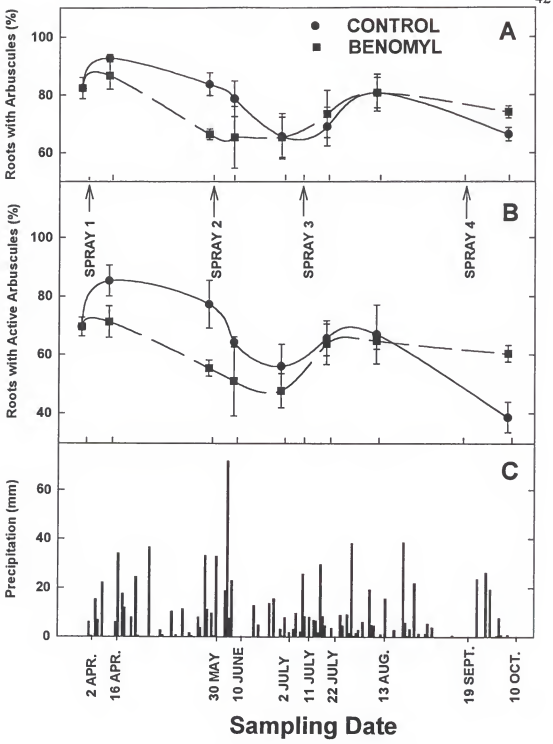

Figure 3-1. Assessment of arbuscular activity in *Panicum chamaelonche* roots from the field site in 1991. (A) Percentage of root length with arbuscules in benomyl-treated and nontreated plots, (B) Percentage root length with metabolically active arbuscules in benomyl-treated and nontreated plots and (C) Precipitation. Each symbol represents the mean of three replicates ± SE.

Table 3-1. Test for homogeneity of slopes for the effect of Benlate® 50 DF applied in the field on percent *Panicum chamaelonche* roots with arbuscules and their activity over time.

	Slope over time	
	Roots with arbuscules (%)	Roots with active arbuscules (%)
Control	-0.104 **	-0.164 **
Benlate	-0.012	-0.010

** indicates slope value is significantly different from 0 at $P \leq 0.01$

application on 2 April (Fig. 3-1A). Over the entire growing season, both the proportion of roots with arbuscules and the activity for benomyl-treated plants did not change significantly, whereas samples from the control plots had significantly negative slopes with time for both arbuscule abundance and activity (Table 3-1). Early in the season ground cover was sparse and the spray was applied directly to the soil. This was paralleled by a short-term decrease in the proportion of roots with arbuscules (Fig. 3-1A) as well as metabolic activity (Fig. 3-1B). As ground cover increased through the growing season, more of the spray was intercepted by foliage leaving less to penetrate through to the soil. Concomitant with this, the differences between treated and nontreated plots disappeared. In late summer, as the plants started to senesce, roots of benomyl-treated plants had more arbuscules and arbuscule activity than nontreated plants. In a concurrent study, no effect of benomyl on shoot P status was observed at samplings taken in June and August. There was no apparent relationship between precipitation, application of benomyl and mycorrhizal response (Fig.3-1C).

Greenhouse Study

Benomyl effects on pine

There were no significant effects of benomyl on inoculated or noninoculated pine biomass (Fig. 3-2A). Phosphorus content of the needles increased over time for all treatments from a mean of 320 mg to 450 mg per plant, but this was not related to the benomyl treatments (data not shown). Similarly, benomyl had no effect on the length or

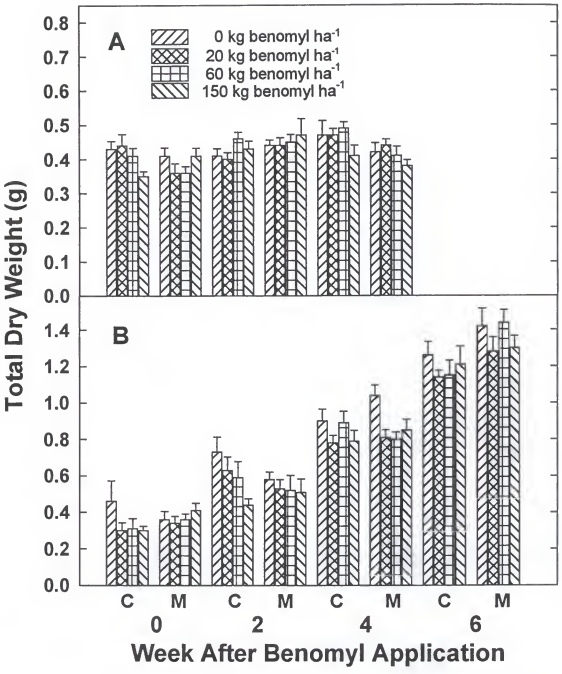

Figure 3-2. Total dry weight of mycorrhizal (M) and nonmycorrhizal (C) plants, (A) *Pinus elliottii* and (B) corn in response to 0, 20, 60 or 150 kg benomyl ha^{-1} in the greenhouse. Each symbol represents the mean of seven replicates $\pm$ SE.

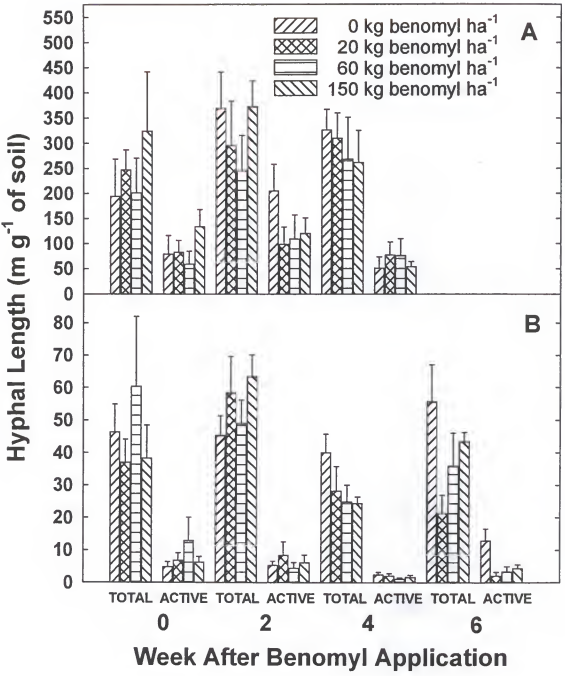

Figure 3-3. Soil hyphal length (total) and activity (active) of mycorrhizal (A) *Pinus elliottii* and (B) corn plants in response to 0, 20, 60 or 150 kg benomyl ha^{-1} in the greenhouse. Each symbol represents the mean of seven replicates $\pm$ SE.

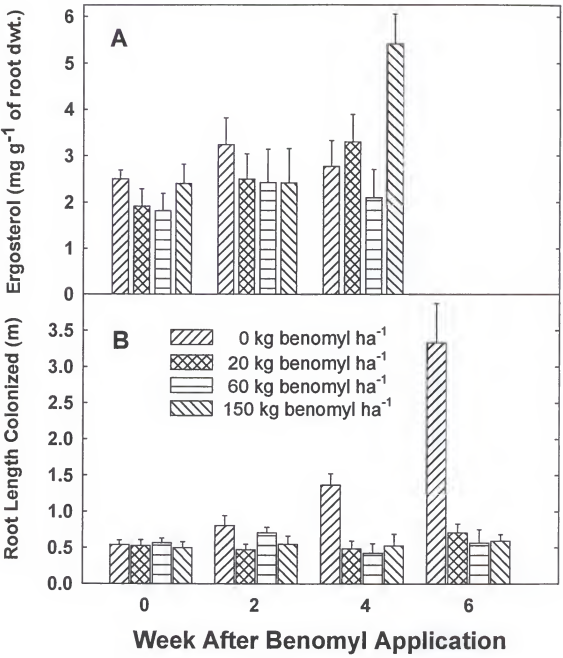

Figure 3-4. Mycorrhizal colonization of (A) slash pine and (B) corn grown in the greenhouse in response to 0, 20, 60 or 150 kg benomyl ha^{-1}. Each symbol represents the mean of seven replicates $\pm$ SE.

viability of external hyphae of the ectomycorrhizal fungus (Fig. 3-3A). There was a difference in EM colonization, as measured by ergosterol concentration, at 4 wk between the 60 and 150 kg benomyl ha^{-1} treatments (Fig. 3-4A); however, this was not repeatable.

Benomyl effects on corn

Benomyl at all concentrations arrested further root colonization by the AM fungus, whereas colonization in the treatment receiving no benomyl continued to increase over the 6-wk period (Fig. 3-4B). There was no dose-related response in colonization. Noninoculated plants remained noncolonized. Total biomass of mycorrhizal and nonmycorrhizal plants was reduced by benomyl by approximately 12% (Fig. 3-2B); however, this was unrelated to the fungicide concentration applied. The length of external hyphae of AM fungi or their viability was not affected significantly or consistently by the different rates of benomyl (Fig. 3-3B). The P concentration of corn leaves decreased steadily throughout the experiment from 3.64 to 0.62 mg P g^{-1} without any evidence of a benomyl effect (data not shown).

Discussion

Corn was used as a substitute for *P. chamaelonche* due to lack of native plant material. Benomyl arrested mycorrhizal development of corn in the greenhouse experiment. This is consistent with the mode of action of benomyl, which entails inhibition of nuclear division by binding to tubulin (Davidse, 1986). There was no dose-

dependent response by the mycorrhizal grass in the greenhouse. The range of concentrations was based on previous values published in the literature (Trappe et al., 1984). The sand used in the greenhouse minimized possible adsorption phenomena that normally occur in field soils. Consequently, all the concentrations tested were above the threshold required to obtain a maximum inhibition of mycorrhizal development. Not only was all of the fungicide readily available, but it was also well above the manufacturer's recommended application rate, which together presumably caused a decrease in plant biomass unrelated to the plant's mycorrhizal status. In agreement with previous literature (Trappe et al., 1984), the effect of benomyl on mycorrhizal pine was neutral although sometimes an increase in growth has been observed (De la Bastide and Kendrick, 1990; Pawuk and Barnett, 1981).

Arbuscules were counted in the field, since they are a distinguishing characteristic of the mycorrhizal fungus, and, more importantly, they represent the site where active exchange of nutrients between the symbionts occurs. The initial decrease in arbuscule activity in the field following benomyl application has been documented in a greenhouse study as well (Sukarno et al., 1993). Since the response to 5 kg benomyl ha^{-1} was minor compared to total colonization, the benomyl application rate was increased. At the last sampling as the plants started to senesce, the increase in arbuscule number and activity in roots of plants treated with benomyl may be due to a reduction in the impact of nonmycorrhizal fungi on plant growth and subsequent mycorrhizal functioning. Low colonization and arbuscule numbers in the greenhouse study made it difficult to obtain a reliable measure of arbuscule abundance to compare to the results in the field. The lack

of AM response to benomyl in the field during most of the growing season may be attributed to the increased interception of the fungicide by ground cover. Although benomyl can enter through leaves, systemic translocation is not as efficient generally as direct application to the target site (Hassall, 1990), in this case, the roots.

Larsen et al. (1994) determined that benomyl applied directly to the leaves of cucumber had little effect on mycorrhizal efficiency, yet when benomyl was applied to the soil, complete inhibition of P uptake by hyphae occurred within 5 d. Although no fungicide effect on fungal alkaline phosphatase activity was found inside the root, the rapid response, nonetheless, suggests some direct influence on uptake or transport mechanisms. Kough et al. (1987) and Thingstrup and Rosendahl (1994) have observed suppressive effects of benomyl on internal fungal enzyme activity in mycorrhizal plants. Although benomyl demonstrated no significant effect on external hyphal length or viability in this study, an inhibitory response has been found in another system (Sukarno et al., 1993).

Benomyl can be an effective tool for inhibiting AM activity in the field; however, researchers need to be aware of the limitations of this approach. The timing of root colonization and initial nutrient contribution to mycorrhizal dependent seedlings can be critical to their survival (Hartnett et al., 1994; Hetrick et al., 1989; Plenchette and Perrin, 1992). Fungicide applications in the field should be timed according to the plant's optimal benefit from mycorrhizas, which, correspondingly, would provide the full impact of the fungicide treatment on mycorrhizal functioning (Gange et al., 1993; Newsham et al., 1995). Furthermore, the frequency of application is determined by fungicide

persistence in the soil, which is variable (Ware, 1992) due to degradation and sorption in different soil environments. In sandy soils where sorption is low, somewhat comparable to the sand used in the greenhouse study, persistence may be longer, assuming leaching does not occur, so that an application every 5 to 6 wk may suffice. Soils with higher levels of organic matter or clay may require more frequent applications or higher concentrations.

The method of application is also critical. Although benomyl is considered a systemic fungicide, translocation from leaves to the active site in the roots appears to be minimal. A soil drench is the optimal method of application (Fitter and Nichols, 1988; Hassall, 1990; Perrin and Plenchette, 1993). Appropriate preparations should be made to accommodate the increasing ground cover as treatments are applied later in the growing season. Tall ground cover may be compensated for by applying a large volume of water to wash the active ingredient to the soil. Benomyl concentrations applied experimentally have ranged from 0.5 to 300 kg benomyl ha^{-1} (Trappe et al., 1984). Treatments of as little as 3 kg ha^{-1} biweekly in a short turf grass setting have been adequate to reduce AM colonization by 80% (Rhodes and Larsen, 1981). As a consequence, the combination of concentration, volume of water used and frequency of application balanced with the environmental conditions should provide the desired reduction of mycorrhizal activity.

CHAPTER 4
MYCORRHIZAS AFFECT PLANT COMPETITION FOR PHOSPHORUS BETWEEN *PINUS ELLIOTTII* and *PANICUM CHAMAELONCHE*

Introduction

Soil fertility largely determines the amount of plant biomass an environment can support (Donald, 1951). In environments with low nutrients, plants are stressed directly by the lack of adequate nutrients, and they survive primarily by stress tolerance mechanisms (Grime, 1979). An environment with more nutrients has the potential to produce more plant biomass, which increases plant growth and also raises the chances that root nutrient depletion zones of two plants will overlap. As a consequence, plant competition for nutrients becomes one of the factors governing plant growth and survival. Environmentally induced stress on a plant, therefore, can be considered a gradient extending from direct physical stress on an individual plant to stress produced biologically by plant interactions (Berkowitz et al., 1995; Grime, 1979).

Autecological studies have extensively documented that mycorrhizas can increase plant tolerance to environmental stresses and contribute to a plant's survival and growth (Sylvia and Williams, 1992). The various mycorrhizal contributions that enhance individual plant health similarly benefit a plant when competing with neighboring plants. Much less research has quantitatively addressed the influence of mycorrhizas on the synecology of plants. Previous studies have demonstrated that mycorrhizas can enhance a plant's competitive ability (Allen and Allen, 1984; Fitter, 1977; Hall, 1978; Hartnett

et al., 1993; Hetrick et al., 1989). The majority of these studies relate to competition between arbuscular-mycorrhizal (AM) plants. To my knowledge, only one study has addressed ectomycorrhizal (EM) effects on plant competition (Perry et al., 1989). Plant competition between EM and AM plants has not been explored specifically.

The goal of this research was to assess the effect of mycorrhizas on the competitive ability of slash pine (*Pinus elliottii* Engelm. var. *elliottii*), which commonly is grown for pulpwood in the southeastern United States. Grasses, among other plants, compete extensively in new slash pine plantations since weed control is practiced infrequently. The specific objectives of the study were to determine (i) if mycorrhizas alter the competitive ability of pine when growing with grass and (ii) how this relationship is modified by phosphorus (P) concentration.

Materials and Methods

Greenhouse Competition Study

All experiments were conducted in acid-washed sand. Acid-washing was accomplished by treating the sand with 25% HCl for 24 h, then draining the acid and rinsing the sand until the pH increased to that of the deionized water being used. Eighty percent of the sand was in the particle size range of 0.160 to 1 mm, and the majority of the remaining portion was larger than 1 mm.

Slash pine seeds were disinfested for 2 min in a 5.25% sodium hypochlorite solution with 0.2 ml Liqui-Nox surfactant (Alconox, Inc., New York, N.Y.) and then rinsed thoroughly with tap water. Seedlings were raised from seed for 2 wk in a growth

chamber [29/23 C° (day/night), with a 15-h light period and irradiance of 1000 μmol m^{-1} s^{-1}] in sand and then transplanted to 50-ml pots (5 cm^2 of surface area) and grown in sand in the greenhouse for 8 wk where they received water only. To inoculate pine, washed roots were dipped in a slurry of rinsed and chopped *Pisolithus tinctorius* (Pers.) Coker & Couch (isolate S106) grown in a liquid suspension culture containing modified Melin-Norkrans liquid medium (Marx, 1969) using glucose instead of sucrose. Roots of control plants were dipped in tap water. After a further 6 wk of growth in 500 ml of sand in Deepots™ (28 cm^2 of surface area; McConkey, Co., Sumner, WA), pine roots were gently rinsed free of adhering sand particles and planted in the appropriate competition treatments as described below.

Grass plants of a dominant competing weed species in the field (*Panicum chamaelonche* Trin.) were obtained from cultures maintained in sand in the greenhouse. Plants were started from seed and vegetatively propagated in 150-ml pots (7 cm^2 of surface area). Two months in advance of the experiment, grass plants were inoculated with pot culture inoculum of *Glomus* sp. (INVAM FL329, formerly FL906) previously cultured on sorghum in pasteurized soil. Roots of plants were washed and the plants transplanted into sand in Deepots™ containing 5 g of soil inoculum (83 spores g^{-1}) located 2-3 cm below the sand surface. Control plants were transplanted into sand without inoculum and received a 5-ml suspension of inoculum filtrate obtained by mixing 60 g of soil from a pot culture with 1.2 L of water and then filtering the mixture through a 10-μm membrane filter. Just prior to the experiment the grass roots were washed as described for the method of pine roots.

Pine and grass plants were sorted separately into three size classes at the start of the experiment. Noninoculated pine had no visual indication of colonization, whereas inoculated pine was heavily colonized. Inoculated grasses had a mean root colonization of 30% at the start of the experiment. There were no significant differences in biomass between inoculated and noninoculated plants at the beginning of the experiment. Intraspecific and interspecific paired combinations of plant species (Table 4-1), inoculated or not, were made by selecting plants from the same size class. Plants were planted together in 500 ml of sand. There were six replications per treatment. Plants were grown in the greenhouse with mean temperatures of 21/34°C (min./max) and a mean photosynthetic photon flux density of 1240 μmol m^{-2} s^{-1} from January to June 1994. A repeat of the experiment was run from May to October 1994 with seven replications. The greenhouse temperature regime was 24/36° (min./max.) with a mean photosynthetic photon flux density of 1490 μmol m^{-2} s^{-1}. The plants were fertilized semiweekly with a solution containing: 660 μM NH_4NO_3, 660 μM $(NH_4)_2SO_4$, 616 μM KCl, 80 μm $MgSO_4$, 54 μM NaFeEDTA, 600 μM $CaCl_2$, 0.25 μM $CuSO_4$, 14 μM H_3BO_3, 40 μM $NaMoO_4$, 2.75 μM MnCl and 1.25 μM $ZnSO_4$. Phosphorus was supplied at either 0.32, 3.23 or 32.26 μM H_3PO_4. In the repeat of this experiment the 0.32 μM H_3PO_4 treatment was replaced with 323.58 μM H_3PO_4, since plant growth was very slow at the lowest P concentration. Soil solution pH was measured from several pots during the experiment by thoroughly watering pots with deionized water and collecting the leachate.

Plants were removed from the pots after 129 d, and the roots of individual plants were separated carefully from each other. The exception was the intraspecific grass

Table 4-1. *Pinus elliottii* (pine) and *Panicum chamaelonche* (grass) treatment combinations planted in the competition study. Two plants were planted per pot. The superscripts "+" and "-" signify an inoculated or noninoculated plant respectively. Pine was inoculated with *Pisolithus tinctorius* and the grass was inoculated with *Glomus* sp. (INVAM FL329, formerly FL906).

Plant Competition Treatments	
Intraspecific	Interspecific
$pine^+$ x $pine^+$	$pine^+$ x $grass^+$
$pine^-$ x $pine^-$	$pine^+$ x $grass^-$
$grass^+$ x $grass^+$	$pine^-$ x $grass^+$
$grass^-$ x $grass^-$	$pine^-$ x $grass^-$

combination where the roots were treated as one unit and then half the value allotted to each plant. Root wet and dry mass were determined. An estimate of root length was obtained using calculations of specific root length (cm root g^{-1} of root fresh weight) for pine and grass from a previous experiment and expressed here as root-length density (cm root cm^{-3} of soil). For grass, root colonization was determined using a gridline-intersect method for the AM treatments (Giovannetti and Mosse, 1980) after clearing the roots for 30 min in 10% KOH and staining in 0.05% aniline blue overnight. For pine, root ergosterol concentration was used as an estimate of EM fungal biomass (Martin et al., 1990; Salmanowicz et al., 1989). Fresh pine roots were washed, ground in liquid nitrogen and thoroughly mixed. A 0.1- to 0.3-g subsample was extracted overnight at room temperature with 5 ml of 100% ethanol. This sample was filtered through a 0.45 μm-syringe filter and then assayed for free ergosterol by high-pressure liquid chromatography (Waters 715 Ultra WISP, Gilson 115 UV detector). Separation was carried out using a C-18 column (Supelcosil™ LC-18; Supelco Inc., Bellefonte, PA) at 40°C with a methanol-water mobile phase (92:8) flowing at 2 ml min^{-1}, with detection at 282 nm.

Shoots were analyzed separately from roots. Shoot wet mass was determined and dry mass was measured after drying overnight at 65°C. The shoots were ground and then ashed at 500°C for a minimum of 4 h. Phosphorus analysis of the shoot tissue was performed using the method of Murphy and Riley (1962).

To compare the competitive abilities of the two plant species, the relative crowding coefficient (RCC; Harper, 1977) was calculated. To avoid subjective pairing of plants between treatments all possible combinations were used to calculate the RCC. The following is a sample calculation of the RCC for grass total dry weight when growing with pine:

$$\text{RCC (shoot P, mg P)} = \frac{\dfrac{\text{grass (interspecific)}}{\text{pine (interspecific)}}}{\dfrac{\text{grass (intraspecific)}}{\text{pine (intraspecific)}}}$$

Data for grass and pine were analyzed separately. To determine if plant competition was affected by the plant species, data for each plant species were subjected to analysis of variance and statistically planned contrasts using the General Linear Model (SAS Institute, Inc., 1989). Data for colonization were transformed to arcsine square roots prior to analysis (Steel and Torrie, 1980). The least-squares means statement within SAS was used to compare means.

Determination of P Uptake Kinetics for Pine and Grass

Pine and grass plants were inoculated with their respective mycorrhizal fungi or noninoculated. Pine plants were grown in Deepots™ for a further 24 wk after inoculation with *P. tinctorius*. Grasses were inoculated 3 wk prior to transferal to 1-L Erlenmeyer flasks by applying to each plant root system a minimum of 20 spores of a mixed culture of *Gigaspora rosea* (INVAM FL224) and *Scutellospora heterogama* (INVAM FL225),

cultured on *P. chamaelonche* in field soil in the greenhouse. The AM fungal species were isolated from *P. chamaelonche* growing in a Spodosol at a field site 21 km northwest of Gainesville. The grasses were replanted together in a 15-L pot of sand. Noninoculated plants were treated in the same manner, except that no spores were added to the roots.

Three grasses and pines, inoculated or noninoculated, were selected, and their roots were gently rinsed free of adhering sand. Each plant was transferred to a single 1-L Erlenmeyer flask covered with aluminum foil. Plants were grown in a growth chamber [29/23 C° (day/night), with a 15-h light period and irradiance of 1000 μmol m^{-1} s^{-1}] in a continuously aerated nutrient solution with the following nutrient composition: 660 μM NH_4NO_3, 616 μM KCl, 800 μM $MgSO_4$, 54 μM NaFeEDTA, 600 μM $Ca(NO_3)_2{\cdot}4H_2O$, 0.75 μM $CuSO_4$, 52 μM H_3BO_3, 120 μM $NaMoO_4$, 8.25 μM MnCl and 3.75 μM $ZnSO_4$. Phosphorus was supplied at 3.23 μM H_3PO_4. The solution was changed semiweekly. At the start of the experiment a minimum 4-wk acclimatization period was given allowing external hyphae to regrow from the colonized roots.

To quantify uptake kinetics, root systems were rinsed with deionized water and placed in additional deionized water for 1 h. One liter of fresh nutrient solution, identical to the one used previously, was added to 1-L acid-washed Erlenmeyer flasks. At the start of the experiment, plant roots were gently patted dry with paper towels, placed in the nutrient solution and weighed. At regular intervals, 23 ml of solution for P analysis were removed and immediately filtered through 0.45-μm syringe filters. The solution was replaced with sufficient deionized water to bring the system back to its original starting weight. Twenty milliliters of sample removed for P analysis were evaporated to dryness.

Twenty milliliters of concentrated HCl were added to the sample and also evaporated to dryness. Phosphorus was determined colorimetrically by a slightly modified procedure of Murphy and Riley (1962). Reagent, quantitatively diluted with deionized water, was added directly to the samples. Since some solutions were at the detection limit, less reagent was added to later samples in order to concentrate them. The resulting P-depletion curve was fit using a curve-fitting procedure (SigmaPlot; Jandell Scientific, San Rafael, California), and uptake calculations were made based upon this idealized curve. Total surface area of roots and hyphae were determined using image analysis software (Mocha; Jandell Scientific, San Rafael, California) or gridline-intersect methods (Giovannetti and Mosse, 1980). The maximum uptake rate, I_{max}, was calculated based on the root surface area and the quantity of P absorbed from the nutrient solution during the first 45 min. The minimum solution concentration from which a nutrient can be absorbed, C_{min}, was considered the asymptotic value where the solution P concentration no longer decreased.

Results

Greenhouse Competition Study

No colonization was found in the inoculated grass plants at the end of the experiment. Therefore these treatments were excluded from further analyses. Also, the ubiquitous EM fungus, *Thelephora terrestris* (Ehrh.) Fr., was found growing on the noninoculated pine ($pine^-$) treatments, but not in the inoculated pine ($pine^+$) treatments. The mean soil solution pH was 4.0.

Pine shoot-P concentration increased in all treatments with increasing level of applied P (Fig. 4-1A). A higher shoot-P concentration was observed in pine$^+$ compared to the pine$^-$ ($P \leq 0.001$). In the interspecific competition treatments where pine was grown with grass, pine$^+$ had an elevated shoot-P content compared to pine$^-$ (Fig. 4-1B). The difference became more apparent with increasing P level. In the treatments where pine was grown with pine, pine$^-$ and pine$^+$ acquired similar quantities of P at all levels of applied P. Total dry weight of pine was not affected by the level of applied P (Fig. 4-1C). Overall pine$^+$ had a higher total dry weight than pine$^-$ ($P = 0.07$) and more so when grown with grass ($P \leq 0.01$). Pines grown with other pines had a lower dry weight than when grown with grass ($P \leq 0.01$). Colonization was also higher in the pine$^+$ treatments inoculated with *P. tinctorius* than in the pine$^-$ treatments that became colonized with *T. terrestris* (Table 4-2).

Similar trends were observed in the repeat of the experiment, although differences were smaller and not always significant. Pine$^+$ grown with grass had a 70% higher shoot-P level compared to pine$^-$ ($P \leq 0.05$), but only at the 32.26-μM P level. The total pine$^+$ biomass was 31% larger than pine$^-$ at 32.26-μM P ($P \leq 0.05$), and only when grown with grass.

For grass shoot-P concentration there was a significant interaction between the level of applied P and the competition treatment ($P \leq 0.05$). At the 32.26-μM P level, the shoot-P concentration of grass when grown with pine$^+$ was lower than when grown with pine$^-$ (Fig. 4-2A). The grass intraspecific competition treatment at this P level was higher than both pine treatments. The shoot-P content at the 32.26-μM P level was also

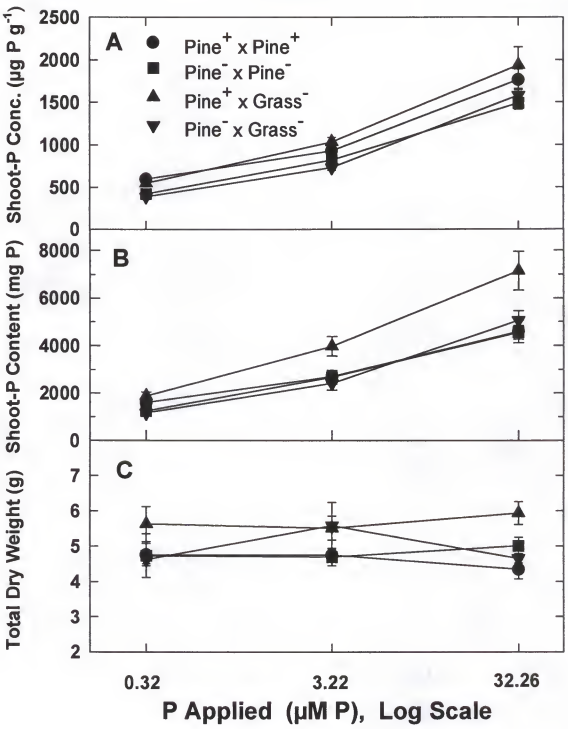

Figure 4-1. *Pinus elliottii* (A) shoot-phosphorus concentration, (B) shoot-phosphorus content and (C) total dry weight in response to different competition treatments and grown at either 0.32, 3.23 or 32.26 μM P for 18 wk. Each symbol represents the mean of six replicates $\pm$ SE. Inoculated grass was not colonized at the end of the experiment and therefore was not included in the analysis.

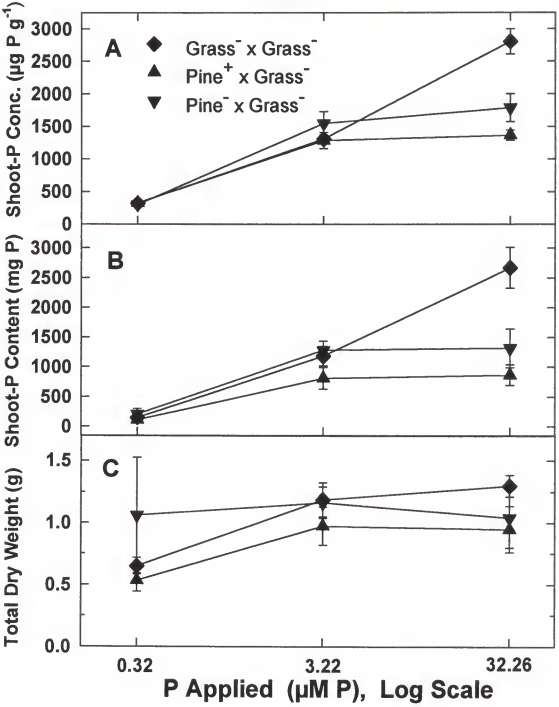

Figure 4-2. *Panicum chamaelonche* (A) shoot-phosphorus concentration, (B) shoot-phosphorus content and (C) total dry weight in response to different competition treatments and grown at either 0.32, 3.23 or 32.26 μM P for 18 wk. Each symbol represents the mean of six replicates $\pm$ SE. Inoculated grass was not colonized at the end of the experiment and therefore was not included in the analysis.

Table 4-2. Ergosterol concentration ($\mu g\ g^{-1}$) of *Pinus elliottii* roots inoculated with *Pisolithus tinctorius* ($pine^+$) or noninoculated ($pine^-$), and grown in combination with *Pinus elliottii* (pine) or *Panicum chamaelonche* (grass) at either 0.32, 3.23 or 32.26 μM P for 18 wk. Each value represents the mean of six replicates $\pm$ SE.

Competition Treatment	Phosphorus added (μM P)		
	0.32	3.23	32.26
$Pine^+$ x $pine^+$	181 $\pm$ 20	282 $\pm$ 34	297 $\pm$ 33
$Pine^-$ x $pine^-$	129 $\pm$ 13	104 $\pm$ 11	150 $\pm$ 11
$Pine^+$ x $grass^-$	192 $\pm$ 8	297 $\pm$ 46	260 $\pm$ 26
$Pine^-$ x $grass^-$	137 $\pm$ 21	139 $\pm$ 12	140 $\pm$ 17

lower in the treatments where grass competed with pine (Fig. 4-2B). Total dry weight of grass was not significantly different at any level of applied P or for any competition treatment (Fig. 4-2C). In the repeat of the experiment there were no differences in shoot-P content between the different grass competition treatments, except at the 322.58-μM P concentration where grass grown with pine had a 39% higher shoot-P content ($P \leq 0.02$) than when grown with another grass. Grass total dry weight at that P concentration was higher in the interspecific treatment with pine than in the intraspecific treatment with grass ($P \leq 0.01$).

Pine$^+$ had a higher root length than pine$^-$ over all treatments ($P \leq 0.001$), even though there were no differences in biomass between inoculated and noninoculated plants at the beginning of the experiment (Fig. 4-3A). Pine root length did not change with the level of P applied. In the repeat of the experiment, response of pine root length did not differ between the competition treatments or between the 0.32- and 3.22-μM P levels (data not shown). When grass was grown with grass, there was an increase in grass root length at the 32.26-μM P level (Fig. 4-3B) which was paralleled by an increase in shoot-P content. At the 32.26-μM P level, grass growing with grass had a higher root length than grass in the interspecific treatments. When grass was grown with pine$^+$, there was an increase in grass root length from the 0.32- to the 3.22-μM P level, whereas grass root length for pine$^-$ was not different between P levels. In the repeat of the experiment, root length increased between the 0.32- and 3.22-μM P levels for grass grown with grass only (data not shown).

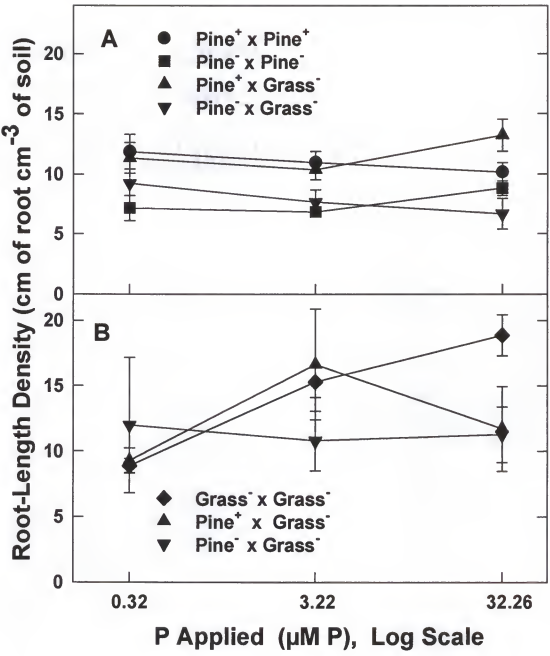

Figure 4-3. Root-length density of (A) *Pinus elliottii* and (B) *Panicum chamaelonche* in different competition treatments and grown at 0.32, 3.23 or 32.26 μM P for 18 wk. Each symbol represents the mean of six replicates $\pm$ SE. Inoculated grass was not colonized at the end of the experiment and therefore was not included in the analysis.

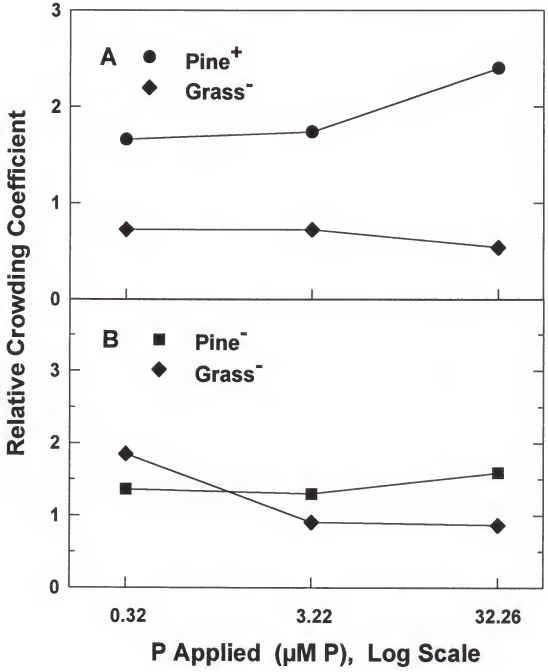

Figure 4-4. Relative crowding coefficient (RCC) for total dry weight of (A) *Pinus elliottii* inoculated with *Pisolithus tinctorius* grown in combination with *Panicum chamaelonche* and (B) noninoculated *Pinus elliottii* grown in combination with *P. chamaelonche* at either 0.32, 3.23 or 32.26 μM P for 18 wk. Each symbol represents the mean of six replicates $\pm$ SE. Mean standard errors were smaller than the symbols and are therefore not included.

Table 4-3. Maximum uptake rate, I_{max}, (μmol P cm^{-2} s^{-1}) and C_{min} (μM P), the minimum solution concentration from which a nutrient can be absorbed, for *Pinus elliottii* and *Panicum chamaelonche* grown in a hydroponic solution containing 0.32 μM P. Each value represents the mean of three replicates $\pm$ SE.

Plant species	I_{max}	C_{min}
Pinus elliottii	0.116 $\pm$ 0.027	0.080 $\pm$ 0.018
Panicum chamaelonche	0.075 $\pm$ 0.016	0.028 $\pm$ 0.014

At each level of P, pine$^+$ had a higher RCC than grass (Fig. 4-4A). In contrast, pine$^-$ was more competitive than grass only above the 0.32-μM P level (Fig. 4-4B). In the repeat of the experiment, grass RCC between 32.26 and 322.58 μM P rose by 279 and 144% when competing with pine$^-$ and pine$^+$ respectively. At this same P level the RCC for pine$^-$ and pine$^+$ dropped by 75 and 27% respectively.

Determination of P Uptake Kinetics for Pine and Grass

Again, no colonization was observed in the grass$^+$ plants, even though I attempted to use indigenous fungi, so the treatment was excluded from analysis. On a root surface area basis, I_{max} was not different between pine$^+$ and pine$^-$; however, I_{max} based on total surface area, which included mycorrhizal hyphae, was much lower in the pine$^+$. Consequently only the values for the nonmycorrhizal pine and grass are shown. A higher I_{max} value was observed for pine, whereas grass had a lower C_{min} value (Table 4-3).

Discussion

Inoculation of slash pine with *P. tinctorius* enhanced P acquisition of pine when grown with nonmycorrhizal grass. This response is dependent on at least two conditions of the experimental design, namely soil volume and nutrient availability. In large soil volumes mycorrhizal fungi are able to enhance plant nutrient uptake by accessing areas beyond the root's nutrient depletion zone. This mechanism is much less important in smaller volumes of soil, such as in this experiment, since roots and fungal hyphae proliferate throughout the pot, essentially making the entire volume a single nutrient

depletion zone. Since an acid-washed sand was used, soluble inorganic nutrients were the only source of nutrients available to both the roots and mycorrhizal fungi. This made the potential ability to utilize nutrients in different forms inconsequential. As a result, differences in P acquisition most likely were related to a combination of differences in absorbing surface area and uptake rates. Although plant density also may affect the outcome of plant competition (Hartnett et al., 1993; Taylor and Aarssen, 1989) this was not tested.

Previous researchers comparing uptake by mycorrhizal and nonmycorrhizal plants have observed a higher uptake rate for mycorrhizal plants (Cress et al., 1979; Karunaratne et al., 1986; Pacheco and Cambraia, 1992). However, these estimates generally are reported on a root weight or root length basis only. If the estimates included hyphal surface area the uptake rates would be greatly reduced for the mycorrhizal plants. In the current study, pine$^+$ and pine$^-$ had similar uptake rates if based on root surface area alone. The lower uptake rate of pine$^+$ compared to pine$^-$, based on total surface area, strongly suggests that hyphal nutrient depletion zones were overlapping. If inadequate mixing of the nutrient solution occurs, the rate-limiting step for uptake would be the replenishment of P absorbed inside the dense mass of hyphae. The P uptake kinetics of nonmycorrhizal pine and grass determined hydroponically in a 0.32-μM P solution were different, but the variability was relatively high due to the low number of replications. The higher I_{max} demonstrated by pine would permit pine to sequester more P than grass, which would give the pine a competitive advantage over grass under the regular fertilization schedule followed here. The lower C_{min} for grass

would be more advantageous where low nutrient concentrations persist over longer time periods, such as in the field but not in this greenhouse study where the time between fertilization was relatively brief. These observations suggest that differences in P uptake kinetics partially may be responsible for the outcome of competition.

Pine and grass root growth rates vary with P concentration, resulting in different absorbing surface areas. The poor relationship I observed between root length and shoot-P content indicates that root length is not the only factor contributing to the pine's competitive interaction with grass. Nonetheless, the increase in root length in the $pine^{+}$ compared to the $pine^{-}$ treatments suggests that mycorrhizal fungi increased pine root length and thereby enhanced nutrient uptake. As a result the competitive ability of pine was increased compared to grass.

The exact nature of the relationship between intensity of competition and resource abundance is still under debate, but it depends on the environmental conditions and plant species involved (Di Tommaso and Aarssen, 1991; Grace, 1995). Tilman (1982) stated that competition increases with decreasing resource availability. An alternate viewpoint is espoused by Grime (1979) who maintained that competition intensity increases with increasing habitat fertility. By definition competition is expressed as effects on plant biomass, survival or reproduction. In this study plants did not demonstrate any dry weight response to P application, which suggests that P was not the only nutrient limiting plant growth. At the 0.32-μM P level plant growth was marginal as a result of inadequate P in the system. Although dry weight was not altered by the different levels of applied P, P uptake by both pine and grass was affected. Since nutrient uptake is part of the

mechanism leading to differences in plant biomass, it is very likely that this would influence plant competition. Differences between P status of grass and pine indicate that P capture by pine reduced the amount of P taken up by grass, specifically at the 32.26-μM P level. Pine, based on its higher RCC, appeared to be more competitive than grass when the two competed with each other. When grown with grass the enhanced P uptake of pine$^+$ corresponded with a larger total dry weight compared to pine$^-$, indicating that inoculation with *P. tinctorius* did alter the competitive ability of pine.

Although not validated in a repeated experiment, grass at 322.58 μM P had a 7.5 or 4.6 times larger RCC than pine$^+$ or pine$^-$, respectively. A change in competitive dominance between the species *Rumex acetosella* and *Poa pratensis* with changes in soil fertility also has been documented (Fowler, 1982). Dual-phasic, P uptake kinetics dependent on solution-P concentration have been found in fungi (Jennings, 1995), plants (Barber, 1972) and in mycorrhizal roots (Cress et al., 1979). If a dual-phasic uptake system exists for each of the plant species, then a higher affinity enzyme system in one of the species could provide a possible explanation for plant dominance based on uptake ability.

When pine was grown with pine, competition was equally intense if the plants were inoculated with *P. tinctorius* or colonized with *T. terrestris*. Since the pine$^-$ plants were not uniformly colonized with *T. terrestris*, I was not able to determine if the EM inoculation treatments substantially altered the intensity of intraspecific competition of pine with and without mycorrhizas. However, in an EM competition study by Perry et

al. (1989), biomass of plants in intraspecific competition (12 trees pot^{-1}) was altered by different EM fungi, indicating that competition intensity varies with the fungal species.

The somewhat reduced response in the repeat of this experiment may be related to temperature differences between the two experiments. The first one ended in June and the second ended in October, which resulted in not only a 2°C higher maximum temperature in the second study, but a longer daily exposure to higher temperatures as well. The mean colonization of $pine^{+}$ in the repeat was 46% lower compared to the first run of the experiment. A decrease of mycorrhizal effectiveness has been observed at temperatures of 34 to 35°C for certain *Pisolithus tinctorius* isolates (Marx et al., 1970) grown on pine, as well as for *Glomus* spp. (Fabig et al., 1989) grown on several grass hosts. The lack of colonization in the grass plants may be related to the high soil acidity (pH 4.0) and the lack of buffering capacity of the sand. Activity of *Glomus* spp. is optimal above pH 5.3 (Abbott and Robson, 1985; Wang et al., 1985).

The conclusion of this study is that *P. tinctorius* can increase P acquisition of pine when grown with grass, which consequently could lead to an increase in competitive ability. The controlled conditions used in this experiment allow the isolation of specific variables that affect a plant's competitive ability. The actual proportion of a plant's total competitive ability contributed by the mycorrhizal component can only be determined under field conditions where soil chemical, physical and biological parameters modify plant interactions and the mycorrhizal response. Yet, our ability to isolate the different components of a plant's competitive ability and determine the relative importance of each

component is limited precisely by the interwoven nature of the plant and soil complex. When this is accomplished, we will be closer to determining the magnitude of mycorrhizal effects on the ecology or economy of an ecosystem.

CHAPTER 5

CONCLUSION

Simultaneously evaluating arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) effects on plant competition for nutrients involves discerning the complex interactions between four different organisms. To do this under controlled experimental conditions requires an understanding of the growth requirements of each species. One of the main problems in my studies was the difficulty in obtaining colonization in the AM grass treatments. This was likely related to the artificial environmental conditions created for the experiments. The main factors distinguishing the field soil from the acid-washed sand were the presence of greater buffering capacity and organic matter in the soil, and differing microbial composition and nutrient regimes. Since *Panicum chamaelonche* was highly colonized in the field compared to the greenhouse, it is likely that modification of one or several of these factors would increase colonization.

Distinguishing the effects of one mycorrhizal type from another can be accomplished by the use of fungicides. In the research presented here the use of benomyl as a tool to control the AM fungal component was tested. The following conclusions can be drawn from this research.

1. Benomyl can successfully inhibit development of an AM fungus under controlled conditions in the greenhouse with no side effects on the EM fungus, *Pisolithus tinctorius*.
2. Early in the season with low ground cover in the field, benomyl caused a slight reduction in arbuscule activity. Later as ground cover increased, systemic translocation of benomyl from shoot to roots of grasses apparently was insufficient to reduce mycorrhizal colonization, even at high benomyl concentrations.
3. Soil drench of benomyl would be a more effective method to place the fungicide directly at the target site, namely the roots.
4. Sufficient water should be used to permit penetration of benomyl into the soil as ground cover increases.

Previous studies have demonstrated that mycorrhizas can enhance a plant's competitive ability. The role of mycorrhizas in competition between EM and AM plants and the effects of different P levels have not been explored specifically. The greenhouse study I conducted to address these interactions yielded the following results.

1. Both the reduction in P acquisition of grass when grown with pine compared to another grass at the 32.26-μM P level amd the higher relative crowding coefficient for total dry weight indicate that pine is more competitive than grass under the conditions tested.

2. Inoculation of slash pine with *P. tinctorius* enhanced both P uptake and total dry weight and hence the competitive ability of pine when competing with nonmycorrhizal grass.
3. When grown in intraspecific competition, no difference was observed in the competitive ability of pine colonized with *P. tinctorius* or *Thelephora terrestris*.
4. The different P levels added did not affect grass or pine biomass which suggests that P was not the only limiting factor to growth.
5. A higher I_{max} value for pine and the lower C_{min} for grass suggest that differing P uptake kinetics can contribute to competitive interactions.

Several additional factors would have to be elucidated to draw conclusions from these results about pine and grass interactions in the field. In a separate field competition study involving slash pine and weeds (primarily grasses) pine growth was substantially decreased in contrast to the greenhouse where pine exhibited a higher competitive ability than grass. In the Spodosol at the field site, organic forms of P are the major source of P, which is released during periodic pulses of nutrient cycling triggered by increases in soil moisture. This contrasts with the inorganic P used in the greenhouse study, which was applied at frequent and regular intervals and thus maintained a relatively consistent P concentration in the system. Also, the buffering capacity of the field soil was absent in the greenhouse, and this would modify plant-soil-microbe interactions by altering nutrient availability and flux. As a consequence differing pine and grass P uptake kinetics expressed in the greenhouse would not necessarily provide the same competitive

advantages in the field. The use of a field soil in subsequent studies would incorporate, at least in part, these effects.

In the hydroponic study on P-uptake an attempt was made to measure the effects of mycorrhizas on P uptake kinetics. The results involving mycorrhizas were inconclusive since mycorrhizal plants with a higher total absorbing surface area demonstrated a lower I_{max} value than the nonmycorrhizal plants. Based on visual observations this is due to extensive hyphal development in the mycorrhizal treatments most likely resulting in overlapping depletion zones of roots and hyphae. Allowing hyphae to regrow for a period of 2 wk instead of 4 wk prior to P uptake measurement probably would have avoided the problem. Although not quantified in the hydroponic study, mycorrhizal fungi may have different I_{max} and C_{min} values from the host plant. If the fungus has a higher I_{max} or a lower C_{min} than the host plant, as well as the competing plant, this would confer a competitive advantage to the host plant.

In the field, root-length density measured down to a depth of 87 cm was several fold higher for grass than for pine. The 500-mL soil volume used in the greenhouse experiment created a root-bound condition which did not fully permit this difference to be expressed. Much of the contribution of mycorrhizas is due to their accessing nutrients beyond the root's nutrient depletion zone; however, since hyphae and roots were able to access nutrients in most of the soil volume in pots, this probably did not contribute a competitive advantage in my study. The spatial advantage mycorrhizal hyphae would provide to a host plant by their presence directly at the site of nutrient mineralization, such as in and around organic matter, also was not expressed. Although external

mycorrhizal hyphae contribute to nutrient uptake and thus plant competitive ability in the field due to their spatial distribution, part of this component could be reduced by hyperparasitism by parasitic fungi and the effects of fungal feeding by Arthropods. Incorporation of a larger nonsterilized, soil volume in future competition studies would allow more components of a plant's competitive ability to function.

The simplification of the soil environment achieved by using an acid-washed sand allowed the isolation of specific mycorrhizal effects that influence plant competition. Ideally, the next step in this process would be to address the competition between mycorrhizal plants in field soil or directly in the field while acknowledging the limitations imposed by the complexity and heterogeneity of field soil conditions.

APPENDIX 1
GROWTH CHAMBER COMPETITION STUDY BETWEEN *PINUS ELLIOTTII* AND *PANICUM CHAMAELONCHE*

Introduction

A competition study involving pine (*Pinus elliottii* Engelm. var. *elliottii*)) and grass (*Panicum chamaelonche* Trin.) was set up to determine: (i) the contribution of mycorrhizal fungal hyphae to total plant P uptake and (ii) the competitive abilities of arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) fungi with respect to each other. This experiment was conducted twice. No colonization was obtained in the inoculated grass treatments the first time this experiment was run, and therefore no competition resulted between plants.

Materials and Methods

The study was initiated as part of a larger field investigation into competition for nutrients between pine and grass at a field site 21 km northwest of Gainesville, Florida. Pine and grass were grown in acid-washed sand and inoculated as in the Material and Methods of Chapter 4. Grasses were inoculated 14 wk prior to the start of the experiment by applying to each plant root system a minimum of 20 spores of a mixed culture of *Gigaspora rosea* (INVAM FL224) and *Scutellospora heterogama* (INVAM FL225) as in the Materials and Methods section of Chapter 4. At the start of the experiment, grass had a mean root colonization of 13%. Pine roots were visually inspected to prevent inclusion

of noninoculated pine colonized by *Thelephora terrestris*. At the start of the experiment plant roots were washed free of all sand and divided into three size classes. Different combinations of plant species, inoculated or noninoculated, were made by selecting pairs of plants from the same size class to create the competition treatments listed in Table 4-1 of Chapter 4. There was a minimum of six replications per treatment.

Growth boxes were constructed with two plant compartments (416 g of dry sand each) on opposite sides of a hyphal compartment (225 g of dry sand). The plant compartments were separated from the hyphal compartment by root-excluding nylon screens (Tetko, Inc., Depew, N.Y.) with a mesh size of either 15 μm for the grass or 40 μm for the pine. The internal dimensions of each plant compartment were 4 x 9 x 11.5 cm (width x length x depth) and 2.5 x 9 x 11.5 cm for the hyphal compartment. Plant fresh weights were measured at the start of the experiment. Eight pine and grass plants, inoculated or noninoculated, were used to determine plant water content and initial P status. After planting, water was added to reach 10% of the soil gravimetric water content and the boxes were then weighed. Deionized water was added to maintain this weight during the experiment. Plant compartments were fertilized separately from the hyphal compartments. In weeks 1, 2, 3 and 6, plant compartments were fertilized three times weekly with 1.4 μmoles P as NaH_2PO_4 along with 10 ml of nutrient solution containing 2.8 mM NH_4NO_3, 2.8 mM $Ca(NO_3)_2$, 2.6 mM KCl, 3.4 mM $MgSO_4$, 230 μM NaFeEDTA, 3.2 μM $CuSO_4$, 221 μM H_3BO_3, 510 μM $NaMoO_4$, 35.1 μM MnCl and 15.9 μM $ZnSO_4$. During weeks 4 and 5 they received 15.1 μmoles P at each fertilization. Starting in the fourth week, hyphal compartments received 8.4 μmoles P. To prevent

massflow of nutrients from the hyphal to the plant compartment, water used to bring the boxes to their original weight was added only to the plant compartment. Plants were grown in a growth chamber with mean temperatures of 23/29°C (dark/light cycle, respectively) and a mean photosynthetic photon flux density of 1000 μmol m^{-2} s^{-1} at plant height. Plant shading was minimized by the distance between plants. Boxes were randomized each time plants were watered in the growth chamber.

Plants were harvested after 62 d. The surface sand layer containing a crusted algal mat approximately 5-mm thick was removed and treated separately. Sand was removed from roots first by shaking and then by rinsing the roots with water. All root pieces were collected. A sample of subsurface sand was retained for further analysis. Plant tissue-P status, colonization and root and hyphal surface areas were measured as in the Materials and Methods of Chapter 4. Root and hyphal surface areas were measured separately for the plant and hyphal compartments. For absorbing surface area calculations involving intraspecifically competing mycorrhizal plants, half of the total hyphal surface area from the hyphal compartment was added to the surface area of the plant on each side. Plant P-uptake rate was calculated as the change in plant P content during the time of the experiment based on combined root and hyphal surface area. The mean total hyphal density for each compartment and treatment was calculated.

Sand for P analysis was thoroughly mixed prior to analysis. A 4.5- to 5.5-g sample in 20-ml, borosilicate vials was treated overnight with 5 ml of concentrated HCl. This was evaporated and 10 ml of 0.1 N HCl were added. Phosphorus was analyzed after 24 h following the procedure of Murphy and Riley (1960). Surface and subsurface soil

samples from the plant and hyphal compartments were treated separately by the same procedure.

Data for grass and pine were analyzed separately. To determine if plant competition was affected by the plant species, data for each plant species were tested by single degree of freedom contrasts using the General Linear Model procedure of SAS (SAS Institute, Inc., 1989). For the contrasts, target plants were analyzed based on mycorrhizal status, type of competition (intraspecific /interspecific) or mycorrhizal status of the competing neighbor plant. Data for colonization were arcsine, square root-transformed prior to analysis (Steel and Torrie, 1980). The least-squares means statement within SAS was used to compare means.

Results

$Pine^+$ exhibited greater plant biomass compared to the $pine^-$ treatments (Fig. A1-1A, Table A1-1). There was no difference between intraspecific and interspecific competition in the $pine^-$ treatments irrespective of the mycorrhizal status of grass. No difference was observed for grass response to intra- or interspecific competition either inoculated or noninoculated (Fig. A1-1B).

Similar to pine biomass, $pine^+$ had a higher plant-P content than $pine^-$ (Fig. A1-2A, Table A1-1). $Grass^+$ had a lower P content than $grass^-$ when competing with both $pine^+$ and $pine^-$ (Fig. A1-2B). No such difference was found in the intraspecific treatments where grass competed with grass.

Table A1-1. Tests for single degree of freedom contrasts for root-length density, plant biomass, plant P content and percent colonization. Each parameter was analyzed separately for grass and pine.

	Root-Length Density ($m\ cm^{-3}$ of soil)	Plant Biomass (g)	Plant P Content ($mg\ P\ g^{-1}$)	Colonization (%)	Uptake Rate ($fmol\ P\ cm^{-2}\ s^{-1}$)
Contrasts for grass:					
$Grass^+$ / $grass^-$	.06	n.s.	.02	.001	.05
($Grass^+$ / $grass^-$) over all pine	.06	n.s.	.03	.02	n.s.
($Pine^+$ / $pine^-$) over all grass	.04	n.s.	n.s.	n.s.	n.s.
Intra- / interspecific	n.s.	n.s.	n.s.	.05	n.s.
Contrasts for pine:					
$Pine^+$ / $pine^-$	$<.001$	.001	.01	.006	.03
($Pine^+$ / $pine^-$) over all grass	$<.001$	$<.001$	.06	.01	n.s.
($Grass^+$ / $grass^-$) over all pine	n.s.	n.s.	n.s.	n.s.	n.s.
Intra- / interspecific	n.s.	n.s.	.07	n.s.	n.s.

Table A1-2. Mean hyphal length density (m cm^{-3} of soil) for each competition treatment presented separately for each compartment (one hyphal and two plant compartments). Hyphal length is made up of the sum of both AM and EM hyphae. Each value represents the mean of a minimum of six replicates ± SE.

Compartment A	Compartment B	Plant Compartment A	Hyphal Compartment	Plant Compartment B
$Pine^+$	$Pine^+$	131.26 ± 13.58	113.12 ± 15.87	131.26 ± 15.58
$Pine^-$	$Pine^-$	94.82 ± 8.69	80.73 ± 12.36	94.82 ± 8.69
$Pine^+$	$Grass^+$	82.40 ± 10.55	63.69 ± 8.01	32.48 ± 6.39
$Pine^+$	$Grass^-$	100.43 ± 7.76	64.32 ± 6.44	21.16 ± 3.69
$Pine^-$	$Grass^+$	75.02 ± 14.88	48.13 ± 11.61	14.47 ± 4.20
$Pine^-$	$Grass^-$	162.27 ± 28.67	65.40 ± 7.71	22.40 ± 6.04
$Grass^+$	$Grass^+$	7.42 ± 1.08	3.75 ± 0.64	7.42 ± 1.08
$Grass^-$	$Grass^-$	6.73 ± 0.82	2.38 ± 0.31	6.73 ± 0.82

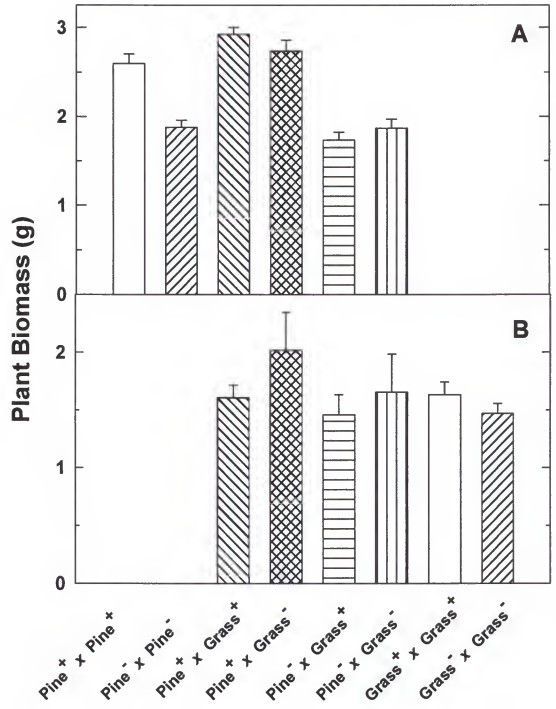

Figure A1-1. Plant biomass for (A) slash pine and (B) grass grown in the growth chamber for 62 d. Each bar represents the mean of a minimum of six replicates ± SE.

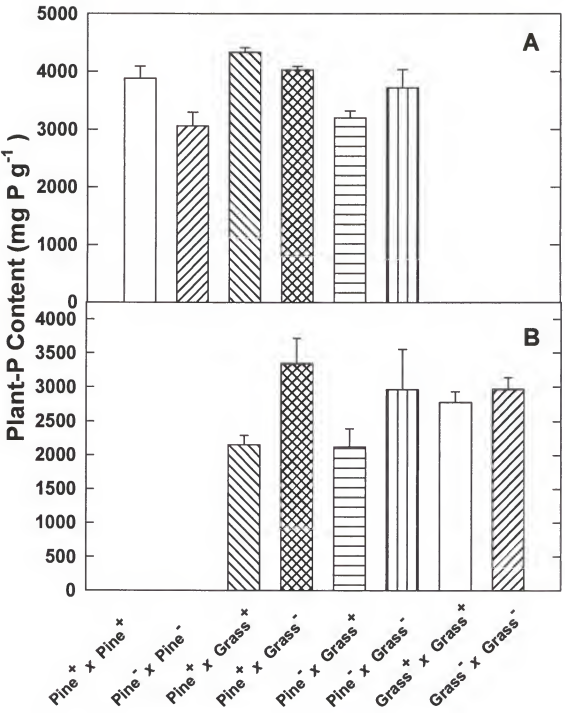

Figure A1-2. Plant-P content for (A) slash pine and (B) grass grown in the growth chamber for 62 d. Each bar represents the mean of a minimum of six replicates ± SE.

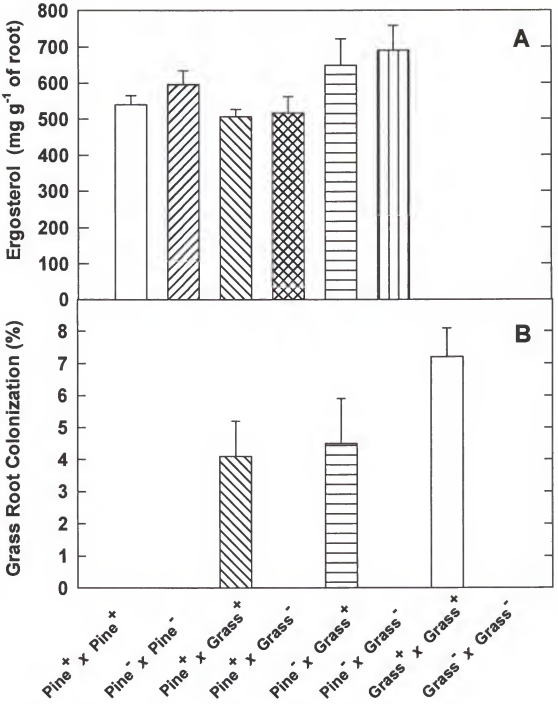

Figure A1-3. Mycorrhizal colonization of (A) slash pine and (B) grass grown in the growth chamber for 62 d. Each bar represents the mean of a minimum of six replicates ± SE.

Pine$^-$ was colonized by *Thelephora terrestris* and had a higher level of colonization than pine$^+$ over all competition treatments (Fig. A1-3A, Table A1-1). In the intraspecific pine treatments, more external hyphae were present in the pine$^+$ than in pine$^-$ treatment (Table A1-2). Grasses were colonized in the grass$^+$ but not in the grass$^-$ treatments (Fig. A1-3B). Lower colonization of grass$^+$ was observed when competing with pine than when competing with another grass. Hyphal length density was higher overall in the pine than in grass. Hyphae contributed between 10 to 16% and 68 to 83% of the total absorbing surface area for grass and pine respectively (data not shown). When total hyphal length was separated by fungal type, AM hyphal length in the plant compartments containing grass was 3.59 ± 0.51 and 3.82 ± 0.68 m cm^{-3} of soil in the pine$^+$ and pine$^-$ treatments, respectively, compared to 7.35 ± 1.09 m cm^{-3} of soil with grass alone. At the lowest hyphal density measured in a compartment, the theoretical inter-hyphal distance was equal to 23.2 μm [calculated using the formula: $2/(L_v\pi)^{0.5}$ from Baldwin and Nye (1974); hyphal length density (L_v) = 238 cm of hyphae cm^{-3} of soil]. The theoretical two-dimensional depletion zone would be approximately 83.1 μm [determined with the formula: $2(Dt)^{0.5}$ (Baldwin and Nye, 1974); assuming a diffusion coefficient (D) = 1×10^{-8} cm^2 s^{-1} and time (t) = 2 d].

Root-length density of pine$^+$ was greater over all competition treatments than that of pine$^-$, independent of intra- or interspecific competition (Fig A1-4A). Grass$^+$ was significantly different from grass$^-$ ($P \leq 0.06$) over all treatments (Table A1-1). The major source of this difference was the higher root-length density of grass$^-$ when competing with pine$^+$ compared to the other competition treatments (Fig. A1-4B).

Table A1-3. Mean soil P content (μg P g^{-1} of soil) for each competition treatment presented separately for each compartment (one hyphal and two plant compartments). Values represent the mean of a minimum of six replicates $\pm$ SE.

Compartment A	Compartment B	Plant Compartment A	Hyphal Compartment	Plant Compartment B
$Pine^+$	$Pine^+$	1758 $\pm$ 99	2543 $\pm$ 224	1758 $\pm$ 99
$Pine^-$	$Pine^-$	2210 $\pm$ 122	2971 $\pm$ 61	2210 $\pm$ 122
$Pine^+$	$Grass^+$	2009 $\pm$ 68	2321 $\pm$ 155	1865 $\pm$ 44
$Pine^+$	$Grass^-$	2091 $\pm$ 238	2622 $\pm$ 204	1722 $\pm$ 203
$Pine^-$	$Grass^+$	2172 $\pm$ 228	3233 $\pm$ 201	2077 $\pm$ 249
$Pine^-$	$Grass^-$	2426 $\pm$ 174	2529 $\pm$ 194	1580 $\pm$ 169
$Grass^+$	$Grass^+$	2142 $\pm$ 261	2732 $\pm$ 255	2142 $\pm$ 261
$Grass^-$	$Grass^-$	1827 $\pm$ 150	2477 $\pm$ 223	1827 $\pm$ 150

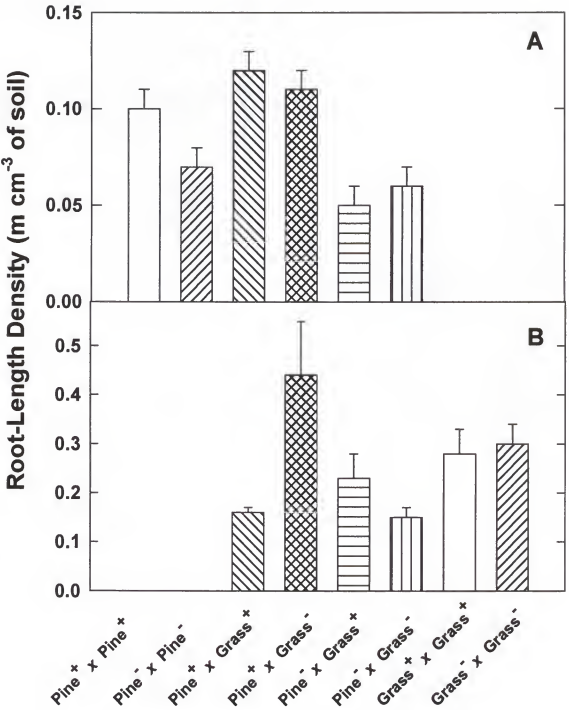

Figure A1-4. Root-length density for (A) slash pine and (B) grass grown in the growth chamber for 62 d. Each bar represents the mean of a minimum of six replicates ± SE.

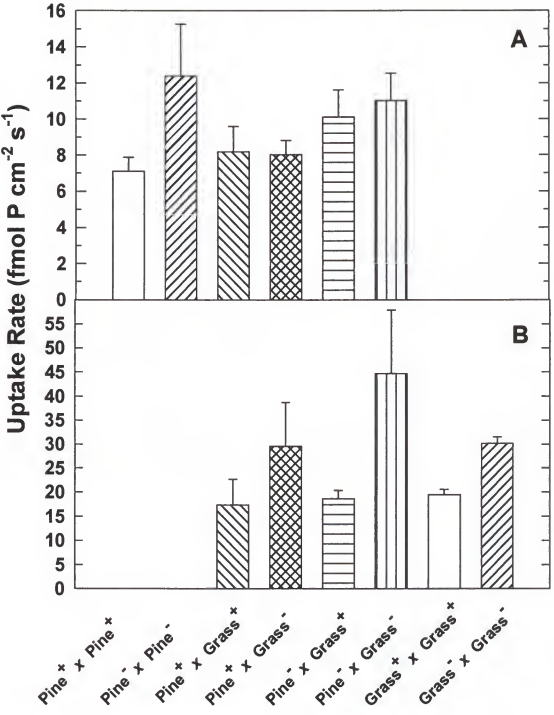

Figure A1-5. Uptake rate of (A) slash pine and (B) grass grown in the growth chamber for 62 d. Uptake rate was calculated on a unit surface area basis which included both root and hyphal surface area. Each bar represents the mean of a minimum of six replicates ± SE.

uptake on a unit surface area basis, which was expressed by the uptake rate calculations. Pine uptake rate was substantially lower then grass which was due to the much higher pine absorbing surface area attributable to external hyphae of mycorrhizal fungi. Since the observed differences are not completely explained by this, it is likely that not all hyphal surface area was active in uptake.

Even though AM fungi of grass, had access to the additional P in the hyphal compartment, grass$^+$ growth was not stimulated. In fact, grass$^+$ had a lower P content than grass$^-$ when competing with pine, inoculated or not. Colonization of grass$^+$ and AM hyphal lengths were lower when competing with pine. It is not clear why this reduction in AM growth occurred; however, it is possible that EM fungi may have exhibited some form of antibiosis. Due to the distance between plants it is less likely that the effect was induced by pine.

A total P budget based on soil and plant P content was not calculated since the pine$^-$ plants became colonized and the AM fungi did not contribute to increased P uptake from the hyphal compartment.

In conclusion, inoculation of pine with *P. tinctorius* enhanced pine P uptake over the pine$^-$ treatment, which was colonized by *T. terrestris*. Competition between pine and grass could not be addressed adequately due to the lack of AM contribution to grass uptake.

Uptake rates for both pine$^+$ and grass$^+$, based on the combined surface area of roots and hyphae, were lower than for the respective noninoculated treatments (Fig. A1-5, Table A1-1). Uptake rate based on root surface area alone increased the difference (data not shown). There were no differences in uptake rates for either pine or grass between their respective intra- and interspecific competition treatments.

Soil-P content for grass$^+$ was higher than grass$^-$, whereas pine$^+$ had slightly less P in the soil than pine$^-$ (Table A1-3).

Discussion

The higher plant biomass and P content of pine$^+$, compared to pine$^-$, were associated with increased root-length density in the pine$^+$ treatments. The mycorrhizal fungus apparently contributed to increased growth of pine$^+$ which is supported by the lower soil-P content in the pine$^+$ compartments. Although pine$^-$ was found to have higher levels of colonization than pine$^+$, the EM fungus, *P. tinctorius*, was more effective than *T. terrestris* at increasing plant growth. It was not possible to determine the additional quantity of P contributed by mycorrhizal fungi since the nonmycorrhizal control was lost when it was colonied by *T. terrestris*. The lower uptake rate in pine$^+$ plants with a higher surface area may have occurred for two reasons: (i) P depletion zones overlapped due to a high density of absorbing surface area or (ii) not all of the surface area used in the calculation was involved in nutrient absorption.

Sample calculations with hyphae alone demonstrate that hyphal depletion zones overlapped even at the lowest hyphal density measured. This would result in less P

APPENDIX 2
COLONIZATION OF *PANICUM CHAMAELONCHE* AND CORN BY DIFFERENT ARBUSCULAR MYCORRHIZAL FUNGI

The objective of this experiment was to determine the ability of different arbuscular mycorrhizal (AM) fungi to form mycorrhizas with either corn (*Zea mays* L. cv. Silver Queen) or *Panicum chamaelonche* Trin. under the conditions in the greenhouse.

Panicum chamaelonche plants were obtained from cultures maintained in sand in the greenhouse. Plants were started from seed collected from the field and vegetatively propagated in 150-ml pots (7 cm^2 of surface area). Corn plants were grown in 150-ml pots for 2 wk prior to inoculation. At the start of the experiment plant roots were washed free of sand. Four plants of each species were inoculated with either a minimum of 0.5 g of onion root fragments colonized by *Acaulospora scrobiculata* (S315), *Gigaspora margarita* (INVAM FL215), *Glomus etunicatum* (INVAM FL312), or *Glomus* sp. (INVAM FL329, formerly FL906), or a minimum of 20 spores of *Gigaspora rosea* (INVAM FL224) or *Scutellospora heterogama* (INVAM FL225). The latter two had been isolated from pot cultures of *P. chamaelonche* originating from the field and grown in field soil in the greenhouse. There were 4 replicates for each treatment. Plants had the same environmental conditions as in the Materials and Methods of Chapter 4 and were fertilized with the same nutrient solution at the 32.26 μM NaH_2PO_4 level. After 9 wk, plants were harvested and colonization was determined using the same technique used in

the Materials and Methods of Chapter 4. This experiment was not repeated. Colonization of corn by AM fungi was lower than that for *P. chamaelonche*, possibly due to the way the two plant species were started. Both plant species in the treatments with two *Gigaspora* spp. and *A. scrobiculata* were more highly colonized than plants in the other treatments, suggesting that these AM fungi may be better suited to the particular combination of soil and greenhouse environment.

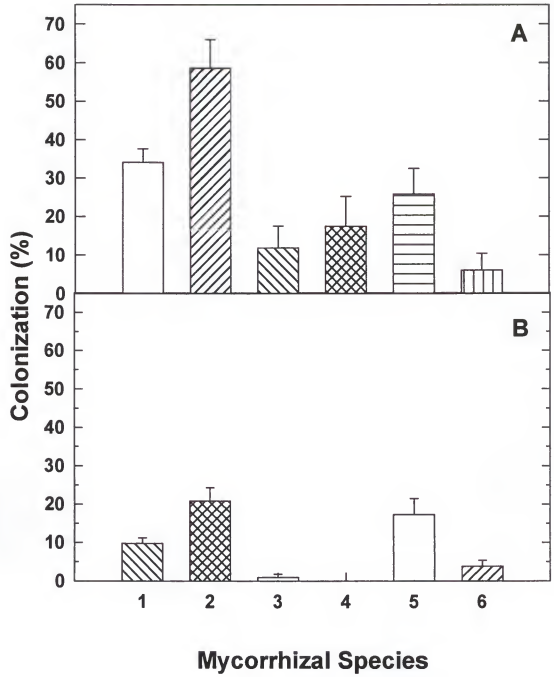

Figure A2-1. Colonization of (A) *Panicum chamaelonche* and (B) *Zea mays* inoculated with root fragments of (1) *Acaulospora scrobiculata* (S315), (2) *Gigaspora margarita* (INVAM FL215), (3) *Glomus etunicatum* (INVAM FL312) or (4) *Glomus* sp. (INVAM FL329, formerly FL906) or spores of (5) *Gigaspora rosea* (INVAM FL224) or (6) *Scutellospora heterogama* (INVAM FL225). Each bar represents the mean of four replicates ± SE.

APPENDIX 3
PHOSPHORUS GROWTH RESPONSE CURVE FOR NONMYCORRHIZAL *PANICUM CHAMAELONCHE*

The objective of the following experiment was to determine the growth response curve of *Panicum chamaelonche* Trin. to phosphorus (P) and to find three appropriate levels of P to apply in the greenhouse competition experiment in Chapter 4. This experiment was only performed once.

Grass plants were started from seed and grown in the greenhouse in 150-ml pots (7 cm^2 of surface area) in sand which had been acid-washed as in the Materials and Methods of Chapter 4. Plants were fertilized semiweekly with nutrient solution containing 660 μM NH_4NO_3, 660 μM $(NH_4)_2SO_4$, 3.23 μM NaH_2PO_4, 616 μM KCl, 80 μm $MgSO_4$, 54 μM NaFeEDTA, 600 μM $CaCl_2$, 0.25 μM $CuSO_4$, 14 μM H_3BO_3, 40 μM $NaMoO_4$, 2.75 μM MnCl and 1.25 μM $ZnSO_4$. After 5 wk, plants were transplanted into 500 g of sand in Deepots™. Fifty 50 ml of the above nutrient solution containing either 0.001, 0.003, 0.010, 0.032, 0.100, 0.316 1.000, 3.162 or 10.000 mg P/L from NaH_2PO_4 was added to each pot biweekly. Pots were leached once per week with deionized water to remove any excess salts and P. The environmental conditions were the same as in the Materials and Methods section of Chapter 4. Plants were harvested after 13 wk of growth. Plants were dried at 65°C and plant biomass and total P were determined using the same methods as in the Materials and Methods section of Chapter 4. Plant response

is shown in Fig. A2-1A and B. The plant growth response at 0.1, 1.0 and 10 mg P L^{-1} would represent plant growth at low, medium and high nutrient regimes.

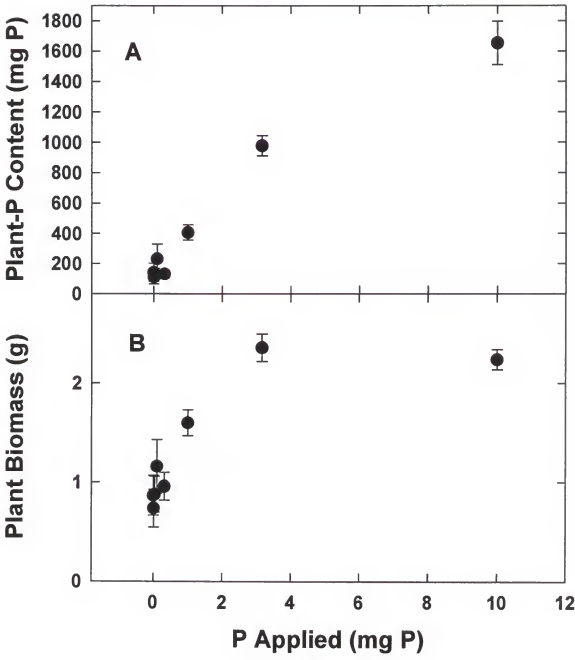

Figure A3-1. Nonmycorrhizal *Panicum chamaelonche* (A) plant biomass and (B) plant phosphorus content in response to 0.001, 0.003, 0.010, 0.032, 0.100, 0.316, 0.1000, 3.162 or 10.000 mg P L^{-1}. Each symbol represents the mean of seven replicates $\pm$ SE.

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BIOGRAPHICAL SKETCH

I was born on April 29, 1958 in Queens, New York. I grew up bilingually learning both Swiss-German as well as English. At the age of 13 my family moved to Switzerland, where I attended the public school system. I completed a four-year degree from the College of Business Administration and Economics in Zurich. In 1980, I returned to the United States where I completed a B.S. in horticulture from The Pennsylvania State University in 1983 specializing in vegetable and fruit production. After various jobs from agricultural consulting to landscaping, I took on a position as assistant winemaker at Bucks Country Vineyards in Pennsylvania. Surrounded by fermenting musts and grape diseases I took an interest in microbiology. Not to forsake plants in the process, I decided to return for graduate studies in soil microbiology. Specifically, my work dealt with tissue-cultured asparagus and mycorrhizas. I completed my M.S. in the Department of Botany and Plant Pathology at the Michigan State University in 1990. From there, to round out my background, I continued towards a Ph.D. in the Soil and Water Science Department at the University of Florida.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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